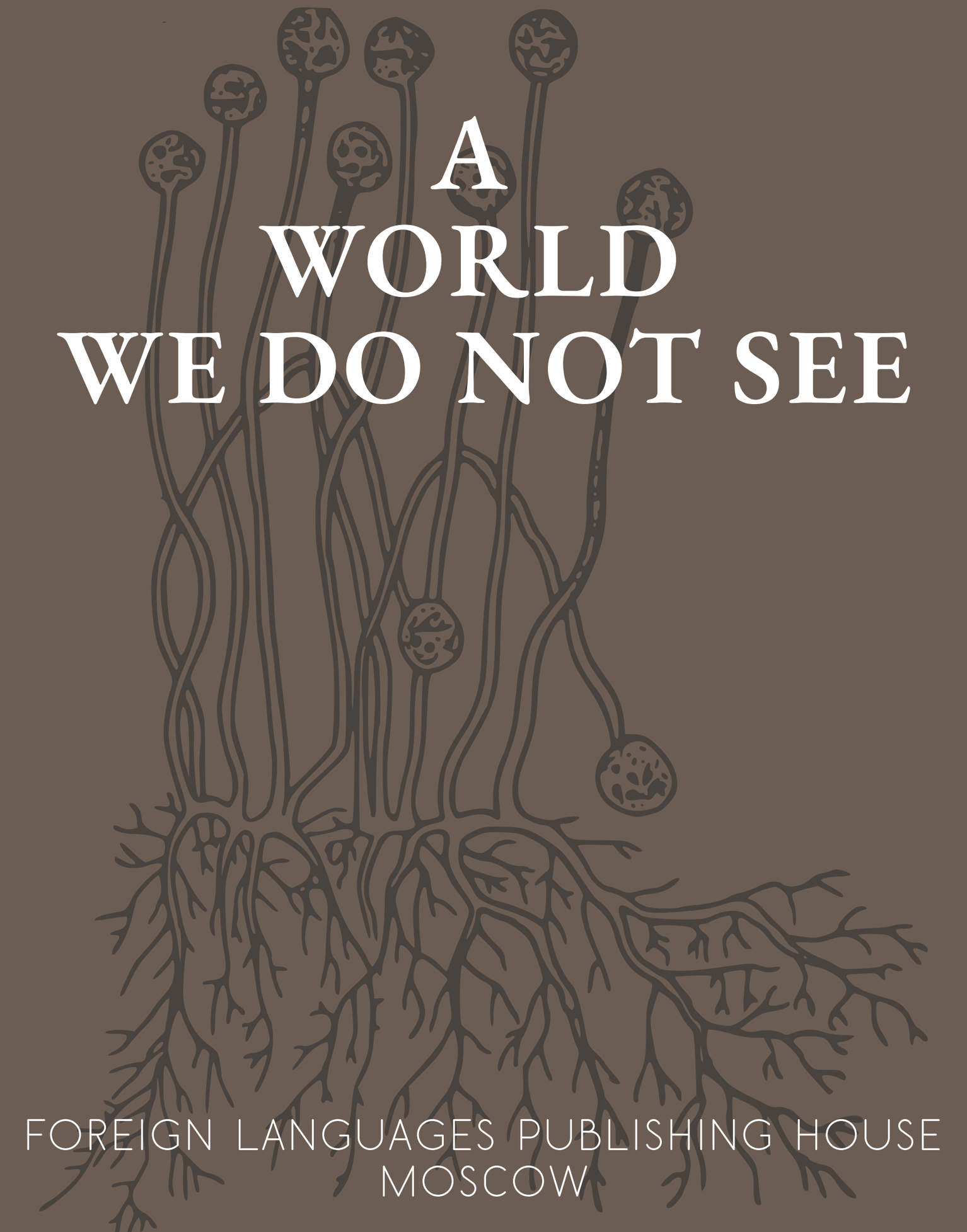
Lev Potkov

A WORLD WE DO NOT SEE



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LEV POTKOV

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CONTENTS

EDITOR'S NOTE 5

INTRODUCTION 7

CHAPTER I. AN HISTORICAL ESSAY 10

CHAPTER II. MICROBES 56

CHAPTER III. SOIL, PLANTS AND MICRO-ORGANISMS 81

CHAPTER IV. THE EARTH'S HISTORY AND MICRO-ORGANISMS . . 103

CHAPTER V. MICROBES IN FOOD AND FODDER 117

- Wine-Making 117
- Production of Alcohol 119
- Brewing 121
- Kvass 122
- Production of Vinegar 123
- Bacteria in Milk and Dairy Produce 125
- Cheese 128
- Bread-Making 131
- Sauerkraut and Pickled Cucumbers 133
- Silage 134

CHAPTER VI. FOODSTUFFS AND FODDER SPOILT BY MICRO-ORGANISMS 136

- Bread 136
- Spontaneous Heating of Grain, Flour, Silage and Hay . 138
- Spoilage Organisms in Milk and Dairy Produce 139
- Putrefactive Microbes 141
- Diseases of Wine 144

CHAPTER VII. PROTECTION OF FOODSTUFFS FROM SPOILAGE MICRO-ORGANISMS 146

- Pickling 148
- Canning 148
- Cold Storage 149

CHAPTER VIII. MICROBES IN CHEMICAL INDUSTRY 152
Butyric Acid 152
Citric Acid 153
Lactic Acid 153
Glycerine 154
Acetone and Butyl Alcohol 154
Microbes and the Textile Industry 154
Finishing Textiles 155
Tanning Leather 156
Fermentation of Cellulose 157

CHAPTER IX. MICROBES HARMFUL TO FUEL, METAL AND WOOD 159

CHAPTER X. MICROBES CAUSING INFECTIOUS DISEASES IN MAN AND ANIMALS 163
Intestinal Diseases *(Typhoid Fever and Dysentery)* . . . 166
Asiatic Cholera 168
Brucellosis 170
Botulism 172
Smallpox or Variola 173
Diphtheria 175
Typhus 177
Recurrent (Relapsing) Fever 178
Infections by Pyogenic Micro-Organisms 179
Forest-Spring Encephalitis 181
Plague 184
Anthrax 187
Tetanus 192
Rabies (Hydrophobia) 194
Tuberculosis 196
Leprosy 199

CHAPTER XI. FIGHT AGAINST PATHOGENIC MICROBES 201

CHAPTER XII. DIRECTED VARIABILITY IN MICROBES 212

EPILOGUA 217

EDITOR'S NOTE

The reading public has always been keenly interested in the world of microscopic beings. And it is not difficult to explain why this is so, for the organisms which can be seen only through the microscope play an exceedingly important role in Nature and in the life of man. It is micro-organisms that are responsible for the rotation of various substances in soil and to a great extent determine its productivity. Therefore, it is only natural that microbes occupy such a prominent place in the works of the great Russian soil scientists V. Dokuchayev, P. Kostychev and V. Williams.

The activity of microbes in water reservoirs is also very important as they are the direct producers of fodder for aquatic fauna.

Today we know that a number of geological formations owe their existence to microbes, which, consequently, are a potent geological factor. Microbiological methods are now widely used in geological survey, particularly, in petroleum prospecting.

Many industries, especially those dealing with fermentation and food processing, are based on the action of micro-organisms. Further development of such industries is wholly dependent on the progress of microbiology.

The readers hardly need being reminded of the fact that micro-organisms are the cause of various diseases of man, animals and plants. Mankind cannot successfully control disease without a thorough study of its agents.

Recently, microbes have been discovered which produce therapeutic substances—antibiotics—whose practical application has proved highly effective, so that a number of diseases formerly considered incurable no longer constitute a threat to humanity. Antibiotics can be used for the treatment not only of humans, but also of animals and plants. The production of antibiotics is an extensive branch of industry.

We could give many more examples illustrating the importance of microbes, but even the few already mentioned are sufficient to draw attention to this group of organisms and make them the object of thorough study.

Russian scientists have made a valuable contribution to microbiology, and the history of the science shall preserve for ever the names of I. Mechnikov, N. Gamaleya, D. Zabolotny, S. Vinogradsky, V. Omelyansky, B. Isachenko, and many other workers. Soviet microbiologists are today further developing the achievements of their teachers on the basis of up-to-date techniques.

It is not easy to describe in popular form the development and successes of microbiology. The book by L. Potkov is an attempt to solve this task. The author had worked long and assiduously, and we hope his effort will not prove in vain and that the book will meet the requirements of the readers.

E. MISHUSTIN,
Corresponding Member,
U.S.S.R. Academy of Science

For my wife

INTRODUCTION

The first descriptions of animals and plants, which laid the foundations of zoology, botany and other biological sciences, were given as early as in the 4th century B. C. For a long time, however, these sciences dealt with phenomena observable with the unaided eye. But there are other phenomena both around and within us, which cannot be understood until we make a study of another world inhabited by living organisms which, although invisible, are, nevertheless, present everywhere and whose vital activity contributes vastly to the changes occurring in Nature, in man and animals.

These minute organisms called microbes (from the Greek *mikros*—small) are of varying length, the average ranging from five to one micron* or even less, and can be seen only through a microscope. The science which deals with microbes is called microbiology.

This book is an attempt to give an idea of the enormous role micro-organisms play in Nature and in the life of man, of microbes that are beneficial to man, and of the ways of combating harmful micro-organisms.

Science first studied microbes which cause infectious diseases. Discoveries in this field revolutionized the attitude towards the origin of infectious diseases and showed the ways of successfully combating them. Speaking in 1899 at a meeting of Russian doctors, dedicated

* A micron is one-millionth of a metre

to the memory of S. Botkin, Ivan Pavlov pointed out the great importance of microbiology and, particularly, of bacteriology, for medicine: "No one will dispute the fact that the great honour of uniting all medicine in experiment belongs to modern bacteriology.... A young and therefore strong branch of science, bacteriology has spread in all the natural and lawful breadth of its idea, breaking up the artificial barriers which still divide other, older branches."*

To give the reader a better idea of the part played by pathogenic microbes in the life of man, the book describes several infectious diseases that used to be the scourge of humanity. Science has made great strides in its fight against these dreaded diseases, but now and again some of them still break out in countries where no preventive measures are taken against infectious diseases.

In the Soviet Union enormous sums are spent on sanitation and on measures for preventing epidemics. Thanks to this solicitude for the health of the population, plague, cholera, smallpox and other epidemics have long become a thing of the past. Great progress has been made in eliminating other communicable diseases.

We shall speak about scientists who not only identified the agents of infections, but also found the means of combating them by utilizing the properties of various micro-organisms. We shall touch upon the activity of soil micro-organisms which contribute to the growth and development of agricultural plants. The reader will be told of the micro-organisms which participate in the physical process of producing fossil fuels (anthracite, coal and peat), coal gas, iron and other ores; he will learn about microbes which are used in the production of alcohol, wine, dairy produce, bread, silage, certain

* I. Pavlov, *Collected Works*, U.S.S.R. Academy of Science Publishing House, Moscow-Leningrad, 1946, Vol. II, p. 354.

chemical preparations, and also in the textile and tanning industries.

Such phenomena as corrosion of metals, decomposition of timber and other building materials are generally known; it would seem at first glance that microbes have nothing to do with all this, but our book will show that the decay and decomposition of certain materials can also be the work of microbes.

The book will give the reader an insight into the multiform activity of micro-organisms which, because of their enormous quantity and varied properties, play a prominent part in the life of both man and animals.

Chapter I

AN HISTORICAL ESSAY

The history of infectious diseases dates back to ancient times. Ancient books tell us about "ravaging epidemic diseases"* known two and even three thousand years B. C. Pest often visited Egypt and the adjacent countries in those times.

In the 6th century A. D. plague swept the Mediterranean countries, from Egypt to Constantinople, where up to 10,000 people died daily. Then through Italy it spread to Europe where it raged for 80 years, reducing many towns and countries to lifeless deserts.

In the 14th century the epidemic gripped a still greater area. Breaking out in China where it took 35,000,000 lives, Black Death spread throughout the world, including the Crimea where the Tatars were besieging Feodosia, then in the hands of the Genoese. Black Death took a heavy toll of life every day. It was then that bacteriological warfare was used for the first time in history. The Tatars catapulted the corpses of men who had died of plague into the town. This bacteriological weapon proved effective: an epidemic broke out and very few of the besieged survived. They flew from Feodosia, carrying the disease to the coasts of the Black and Mediterranean seas, to Constantinople and Italy. Gabriel de Mussis,**

* Cf. H. Heser, *The History of Epidemic Diseases.*

** Gabriel de Mussis, a young lawyer of Piacenza, lived in the Crimea between 1344 and 1346, before the epidemic broke out

one of the survivors, wrote that of 1,000 men on his ship only ten reached Genoa, and that half the population of Genoa died after their arrival. The same happened in Venice and in other towns.

Fig. 1 Plague in ancient Rome

Giovanni Boccaccio painted a gruesome picture of the bubonic plague he witnessed in Florence in 1348, of which patients died on the third day after the disease broke out. Doctors, drugs, precautionary measures were all in vain. "And thereagainst no wisdom availing nor human foresight—nor yet humble supplications, not once but many times both in ordered processions and on other wise made unto God by devout persons.... Very many, both men and women, abandoned their own city, their own houses and homes, their kinsfolk and possessions ... this tribulation had stricken such terror to the hearts of all, men and women alike, that brother foresook brother ... and oftentimes wife husband; nay (what is yet more extraordinary and well nigh incredible), fathers

and mothers refused to visit or tend their very children, as they had not been theirs.... The condition of the common people (and belike, in great part, of the middle class also) was yet more pitiable to behold, for that these ... sickened by the thousand daily and being altogether untended and unsuccoured, died well nigh all without recourse ... nor was it only one bier that carried two or three corpses, nor did this happen but once; nay, many might have been counted which contained husband and wife, two or three brothers, father and son or the like. And an infinite number of times it befell that, the priests going with one cross for some one, three or four biers, borne by bearers, ranged themselves behind the latter,* and whereas the priest thought to have but one dead man to bury, they had six or eight, and whiles more ... the thing was come to such a pass that folk recked no more of men that died than nowadays they would of goats.... The consecrated ground sufficing not to the burial of the vast multitude of corpses aforesaid, which daily and well nigh hourly came carried in crowds to every church ... there were made throughout the churchyards, after every other part was full, vast trenches, wherein those who came after were laid by the hundred ... it is believed for certain that upward of a hundred thousand human beings perished within the walls of the city of Florence."**

Within 9 years the epidemic laid waste France, and killed nine-tenths of the population of England.

Then, through Poland, plague spread to Russia where in seven months of 1349 it carried off more than half the population. In Glukhov, Belozersk and other Russian towns not a single person survived.

Plague continued periodically to ravage Europe up to the end of the 14th century.

* I.e., the cross.—(*Note by J. Payne.*)

** Giovanni Boccaccio, *The Decameron*, translated by John Payne.

In 1402 it penetrated to Iceland and wiped out two-thirds of the population.* During that period over 25,000,000 people in Europe—more than a quarter of the population—died of plague.

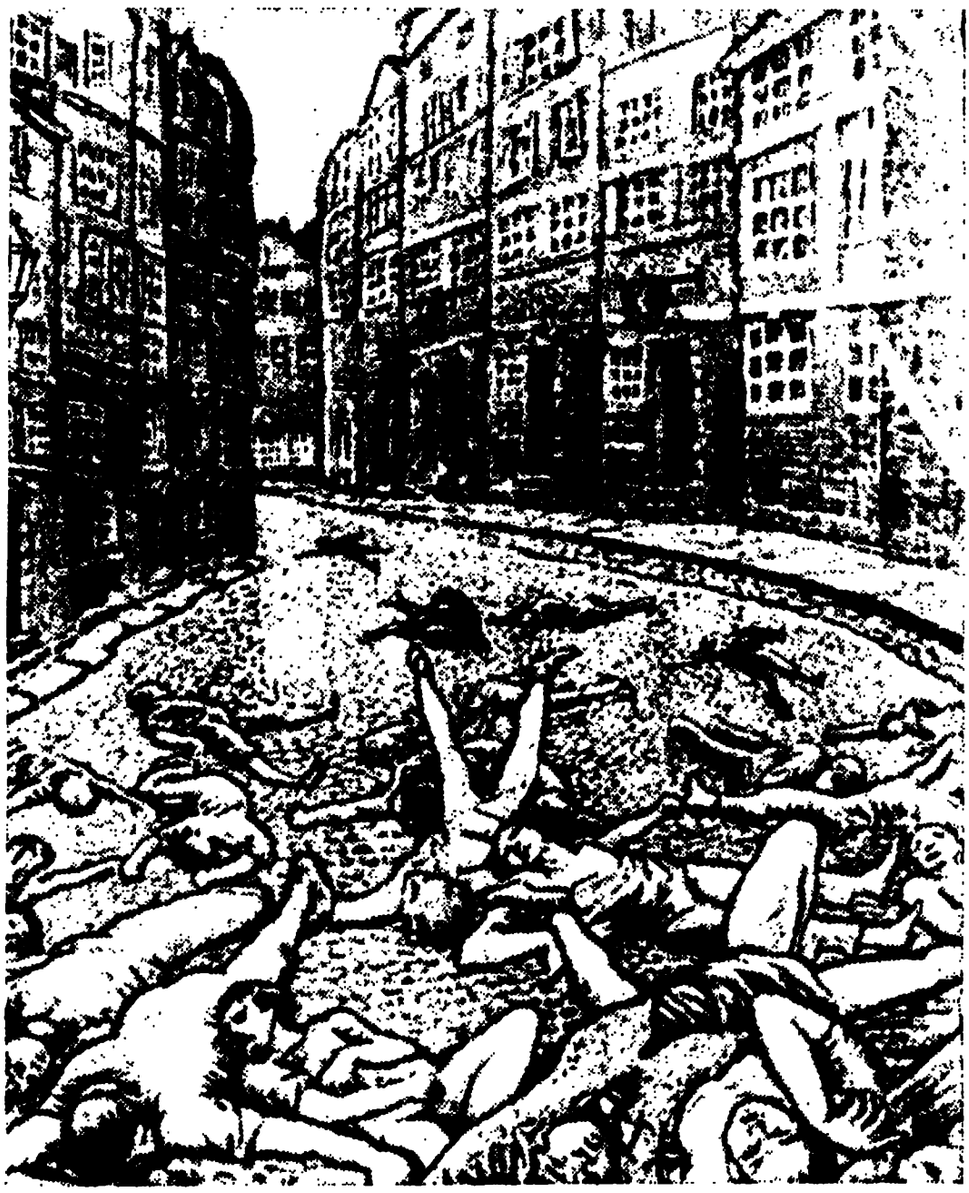

Fig. 2 Plague in 14th-century London

In his *Yemelyan Pugachov* the writer Vyacheslav Shishkov gives the following description of an epidemic in Moscow at the end of the 18th century.

"At the end of 1770 plague, or Black Death as it was called, spread from Turkey to Moscow.

"This fearful visitor had not appeared in Moscow

H. Heser, *The History of Epidemic Diseases.*

since the time of Tsar Alexei, and medicine was powerless to allay the epidemic.

"Afanasy Shafonsky, Chief Doctor of the Land Hospital ... reported to the aged Fieldmarshal Saltykov: 'This disease has been imported here with the war booty....'"

People died en masse. The present Nikolai Ostrovsky Street in Moscow formerly bore the name of Dead Lane, because during the epidemic in 1770-72 not a single person was left alive in that lane.

To quote Shishkov further: "The streets were strewn with the dead, especially in the outskirts. A rumour spread that the authorities had bribed the leeches to exterminate the poor folk. The doors of all bath-houses had long been sealed. Market places were shut down. Famine began. Those who could move tried to sneak out of Moscow, sometimes taking their sick with them. Some even managed to escape from hospitals and quarantines. They were chased, caught and beaten up."

Despairing of all earthly hope, the people sought salvation in religious processions, which tended further to spread the epidemic. Now one, then another would drop out and die on the spot. The epidemic took a toll of 75,000 people, nearly a quarter of Moscow's population.

Another infectious disease known four centuries B. C. was typhus which had been imported to Greece from Ethiopia and Egypt. Some scientists believe that the disease originated in Africa and from there spread to other countries.

In the 16th century typhus accompanied the incessant wars of conquest waged by the Ottoman Empire, and during the Thirty Years' War in the following century it spread all over Europe. The disease raged in Germany and from there penetrated further south, to France, and to the Scandinavian countries, in the north.

The ceaseless wars and famine in Europe contributed to the spreading of typhus, and the disease was therefore often called "war" or "famine" typhus.

Typhus was brought to Russia during the Patriotic War against Napoleon in 1812. More soldiers on both sides perished of typhus than were killed in battle.

In the twenties of the past century Russia had another unbidden visitor—Asiatic cholera. First it came from

Fig. 3. The 1770-72 plague epidemic in Moscow

India and some time later from China. In 1830 a cholera epidemic spread throughout many Russian towns and villages and carried away more than 40,000 lives.

This calamity, aggravated by famine and the misdeeds of tsarist officials, led to unrest and revolt, which tsarist historians called "cholera riots."

Cholera made 5 rounds of Europe during the 19th century, visiting Russia 50 times. Each appearance of the disease took an enormous toll of lives.

Smallpox or variola was another antique scourge which used to be a frequent visitor in all countries.

In ancient times man attributed all natural phenomena to supernatural forces. That gave rise to belief in gods, in good and evil spirits, who were supposed to govern the life of people. But even then scientists tried to find an explanation of various natural phenomena, including the origin and spread of infectious diseases. Thus, Hippocrates, the great physician of the ancient world who lived between 475 and 380 B.C., explained the outbreaks of epidemics as the result of infectious polluting exhalations, which he called miasma. He believed miasma to come from rain, since rain water soon becomes putrid, causes colds, coughs, etc.

Hippocrates' idea of the causes of infectious diseases was for many centuries considered to be correct and the word "miasma" occurs in scientific literature as late as the 19th century.

Even in that century some scientists, e.g., Heser,* attached much importance to "bad air," earthquakes, solar eclipses, floods, eruptions, comets and other natural phenomena, believing them to cause epidemic diseases.

Thucydides, a Greek historian who was contemporary of Hippocrates, was nearer the truth when he explained the epidemic he witnessed (probably, plague) by infection through live *contagia* (*contagium vivum*).**

Varro, a Roman sage who lived in the first century B.C., considered that "the grave bog disease (probably, malaria) was caused by very small animals, unseen with the eye, which grow in a bog and enter man by way of the nose and mouth."

The Italian Girolamo Fracastor (1483-1553) developed further Thucydides' idea in his book *On Contagion and Contagious Diseases* (1546). He held that infectious diseases were transmitted first through direct contact,

* H. Heser, *The History of Epidemic Diseases.*

** The *contagium* was supposed to be a living infectious factor spreading to healthy people through contact with the sick.

when a healthy person touched a sick being; second, through the use of, or contact with, contaminated objects; and third, through the air—at a distance.

The above shows that though Fracastor was not aware of the existence of micro-organisms, he was not far from the truth.

All this, however, was mere guesswork and although their assumptions revealed great penetration and genius, the ancient and mediaeval sages could not prove their theories.

The science treating of microbes began to develop only after the invention of the microscope—along with other natural sciences.

The magnifying glass was known from ancient times. Nero, for instance, liked to watch gladiators fight through an emerald in his ring.

Spectacles for reading were invented in Italy in the 13th century. At about the same time Roger Bacon made a vain attempt to combine several magnifying glasses in order to obtain an apparatus of maximum magnifying power.

More than three centuries later Galileo, the great thinker and successor of Copernicus and Giordano Bruno, improved the simple spyglass by substituting a plano-convex and a plano-concave lenses for the bi-convex ones. By observing the sky through his spyglass Galileo converted it into a telescope. His observations and intuition enabled the brilliant Galileo to combine the data collected before him into a harmonious system shedding the first rays of light on the gloom of the universe surrounding mankind.

While experimenting with his telescope Galileo unwittingly increased the distance between the lenses so that the glass began to magnify objects at close range. By training his glass on a window Galileo saw on the window-pane a huge fly, and in this way the idea of the microscope was born.

Fig. 4 Drebel's microscope

Although Galileo worked for fifteen years perfecting his microscope, his was not the prototype of the modern instrument. The modern microscope was probably developed from the one constructed by Drebel, a well-known physicist of the 17th century (Fig. 4).*

Athanasius Kircher, a German Jesuit, constructed his "flea-glass," as he called it, in the middle of that century; it was a primitive microscope permitting a detailed examination of a flea's body. With the help of his glass Kircher observed "minute living worms" in putrid meat, milk, cheese and the blood of sick persons. What Kircher observed through his glass, which magnified from 40 to 100 times, is hard to say, but it could not have been bacteria which are less than 4 or 4.5 microns. Kircher asserted he had seen "worms" in smallpox pustules, although we know now that the smallpox causative organism is several times smaller than the minutest bacteria and can be seen only through modern super-microscopes and even then it must be specially prepared. The important thing is that it occurred to Kircher to look for a live pathogen of smallpox. He also claimed to have seen "worms" in the blood of plague patients.

While in Holland Peter the Great of Russia sailed in

* Cf. S. Sobol, *The History of the Microscope and Microscopic Research in 18th-Century Russia*, U.S.S.R. Academy of Science Publishing House, 1949.

a yacht along the Schie on a May day in 1698. On arriving at Delft he ordered a stop and expressed a desire to see Anton van Leeuwenhoek whose wonderful discoveries were well known to the Tsar.

Leeuwenhoek was a petty official in Delft: his duties included opening the office in the morning, tidying it up, heating the stove and locking up for the night. Leeuwenhoek devoted his free time to optics. He ground lenses so well that his magnifying glass consisting of a bi-convex lens no bigger than a pinhead magnified from 160 to 200 diameters, which was far in excess of all that was achieved with complex microscopes constructed by his contemporaries, specialists in optical instruments (Fig. 5). It was not greed that prompted Leeuwenhoek to master his art and produce the best magnifying glasses of his day but the desire to fathom the mysteries of the invisible world.

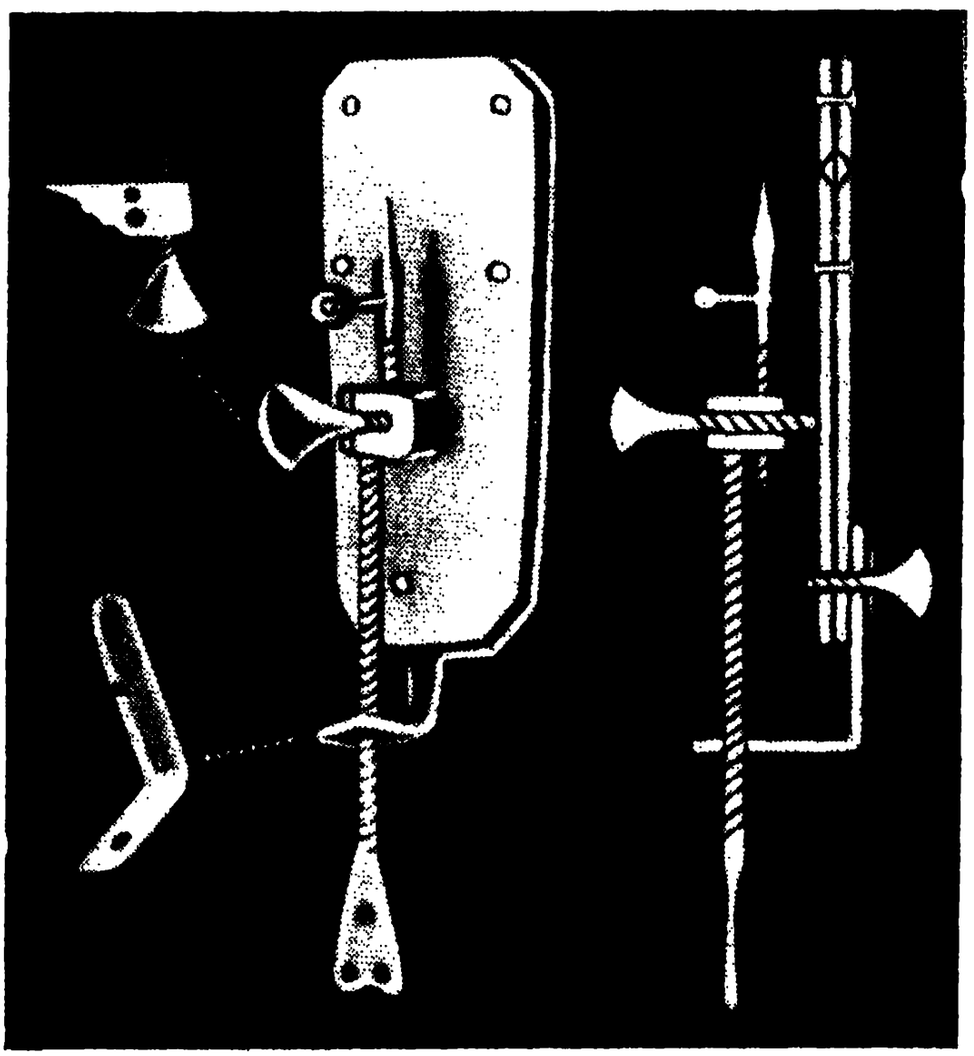

Fig. 5 **Leeuwenhoek's magnifying glass**

Leeuwenhoek presented himself before the Russian Tsar, bringing some of his instruments on board. He demonstrated to him the "circulation of blood in the eel's tail" and some other of his discoveries.

Before parting the Tsar shook hands and thanked Leeuwenhoek for the opportunity of examining such minute objects, which "probably included infusoria and other 'animalcules' in water, and the 'spermatic animalcules,' i.e., spermatozoa, first described by Leeuwenhoek in 1677, as well as the eyes and mouth parts of insects, the scales of butterflies, a louse, a bug, a flea and a gnat. He even showed Peter the minute 'animalcules' in dental tartar."*

In his report to the Royal Society in London, the highest scientific institution of those days, Leeuwenhoek wrote: "There are more of them (bacteria—*L.P.*) in my mouth than there are people in the Kingdom of the Netherlands."

Leeuwenhoek did not understand the importance of the "tiny beasties" he had discovered, he merely described what they were like. But his merit lies in founding the science of micrography—the description of minute objects that cannot be perceived by the naked eye.

Leeuwenhoek's magnifying glasses were followed by microscopes of various types, the one by Robert Hooke (Fig. 6) being the most interesting for the fact that it introduced a new feature—artificial lighting. In this microscope the rays of an oil lamp passing through a glass sphere filled with water fell on a thick plano-convex lens.

Unfortunately, the microscope was then considered a plaything. Linnaeus, the famous botanist, for instance, regarded it as a useless gimcrack and Voltaire remarked sarcastically that the "microscopists" were studying "spots in their own eyes." Therefore optical masters

* Quotation from S. Sobol, *The History of the Microscope and Microscopic Research in 18th-Century Russia,* 1949.

devoted more attention to adorning their microscopes than to improving their optical properties—the magnifying power and distinctness of the image. A large variety

Fig. 6 Robert Hooke's microscope

of microscopes were produced, from cheap cardboard instruments to expensive silver ones.

In the second half of the 18th century Prof. Leonhard Euler of the Academy of Science of Russia and his pupil N. Fuss made the first attempt to free the microscope of

the grave faults common to the instruments of that time, which produced a blurred and coloured image.

The task of realizing Euler's idea was entrusted to Ivan Kulibin, chief of the Precision Instruments Workshop of the Academy of Science of Russia, who with his assistant I. Belayev and his pupil I. Shersnevsky, constructed the first achromatic microscope, free from the colouring of the object which was due to the diffraction of the rays of light.

Further development of the microscope along these lines has brought it to its present highly developed state (Fig. 7). Modern light microscopes give us a clear and naturally coloured image of objects as small as 0.2 micron.

About 20 years ago the electron microscope was invented which is capable of magnifying objects from 100,000 to 200,000 times. In the electron microscope the object under examination is projected on a screen or photographed on a plate.

Nowadays new methods of microscopy have been developed—the fluorescence and phase-contrast. The fluorescence (luminescence) microscopy is based on the property of objects to become luminescent (the colour depending on the object) under the influence of ultraviolet or visible short-wave rays.

Some micro-organisms become luminescent in a very slight degree and in such cases the method of secondary (or induced) fluorescence is used. This is done with the help of so-called fluorochromes—weak solutions of dyes capable of inducing fluorescence, such as acridin (orange and yellow), auramin, neitralrot, primulin, tripaflavin, fluorescein, and some others.

After being treated by these dyes the preparations become fluorescent when acted upon by ultraviolet or visible blue short-wave rays. The colour the preparations acquire depends on the properties of the microbe and of the fluorochrome.

The luminescence (fluorescence) method enables us to examine both live microbes and preparations of dead microbes.*

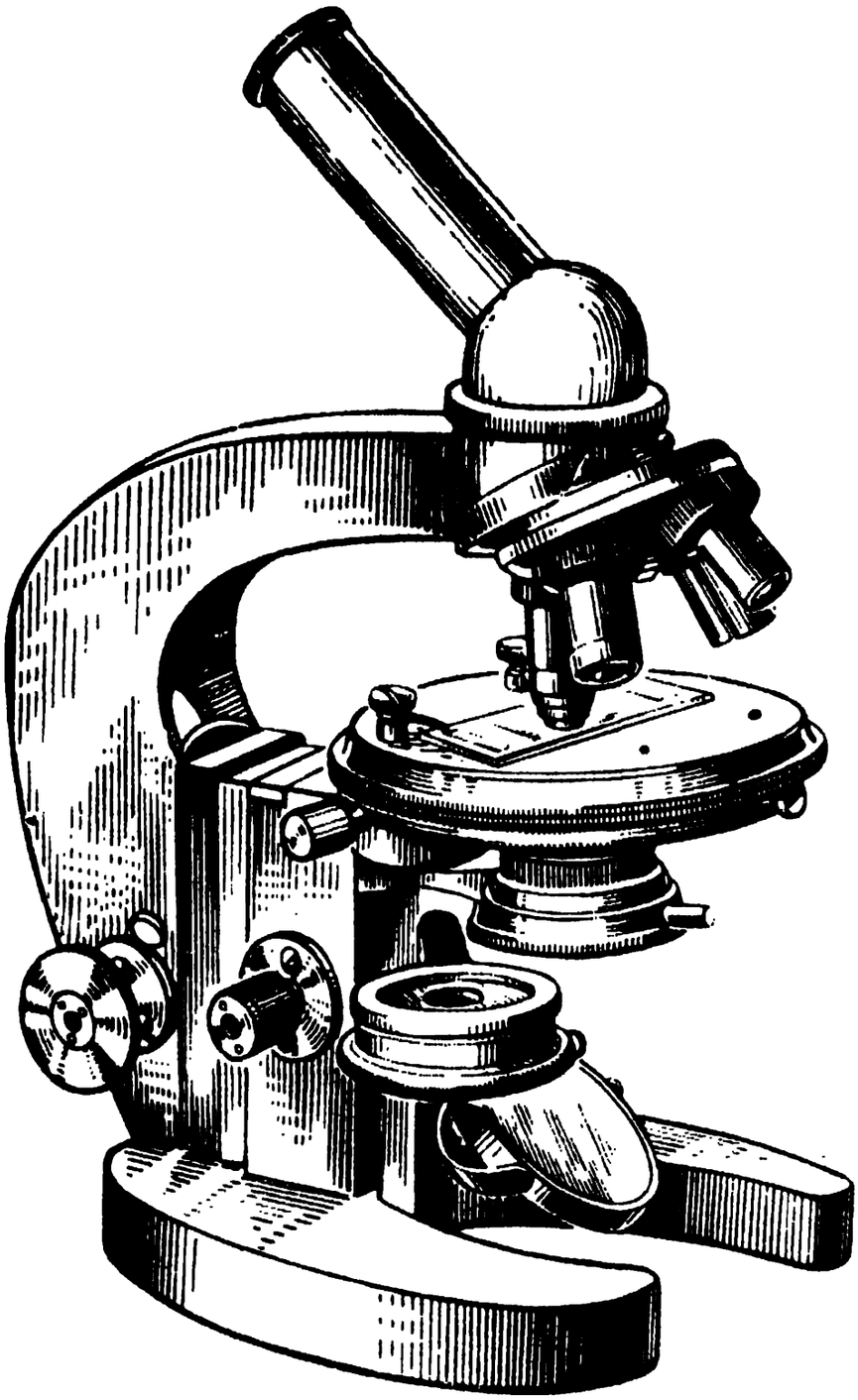

Fig. 7 Modern optical microscope

The phase-contrast microscope invented a few years ago makes it possible to examine the structural details of live microbes without previous dyeing.

* M. Meisel, *Optical Methods of Studying the Structure and Functional State of Yeast Organisms. Problems of Microbiology in Wine-Making and Viticulture,* Moscow, U.S.S.R. Academy of Science Publishing House, 1952.

Thanks to phase-contrast and, particularly, luminescence microscopy, we are able to examine the structure of micro-organisms, such as yeast cells, to distinguish and study the vital functions of the various parts of a live cell, the so-called organoids, which play the part of organs in unicellular organisms. While examining live yeast cells stained with a fluorochrome through the luminescence (or phase-contrast) microscope we can quickly and accurately define the transition of yeast organisms from a state of rest to that of active growth and reproduction.

These methods allow us to discern the depression of vitality in yeast cells, and other abnormal changes in live microbes, to discover nitrogen or vitamin famine, and other defects in the nutrition of the microbes. In addition, the luminescence method involving the use of fluorochromes provides a means of distinguishing live yeast cells from dead ones.

Such has been the progress of microscopy in later years that now we can study even filter-passing viruses, the minutest micro-organisms which are far beyond the range of ordinary light microscopes, being only from 160 to 8 millimicrons in length.

Although early in the 18th century microscopes had but a small magnifying power, they promoted the development of the important branch of biology springing from Leeuwenhoek's discoveries in the preceding century. This discovery of an invisible world awakened people's minds and men began to speculate on the possible role of the "animalcules."

Thus, at the beginning of the 18th century Lancisi tried to explain the cause of malaria by the activity of "invisible tiny animals" and, when in 1721 plague broke out in Toulon and Marseilles, Vallisneri offered the same explanation.

Plenciz, a Viennese doctor, made an interesting conjecture in 1762: "Just as a definite plant can grow out of

Danilo Samoilovich

seeds of the same plant, smallpox can arise only out of smallpox seeds and scarlet fever, out of scarlet-fever seeds."*

The observation of putrid foodstuffs led Plenciz to the conclusion that putrefaction sets in only when embryos of worm-like beings begin to develop and reproduce in the product.

For many years, Danilo Samoilovich, a Russian doctor of the latter half of the 18th century, examined under

* Marci Antonii Plenciz, *Opera Medico-Physica*, p. 22. *Tractatus II de variolis. Cap. I-III; Tractatus III, sectio IV. De scarlatinae causis, XXXI*, p. 64.

the microscope the corpses of people who died of plague. In 1792 he published a book entitled *A Short Description of Microscopic Research into the Essence of Ulcerous Toxin*, the fundamental idea of which was that "ulcerous toxin contains no reptiles ... but is made up of some very original matter which hitherto nobody knew anything about and which I have studied with the assistance of precise microscopic and other observations." Samoilovich did not only try to discover the causative organism of plague, he was the first to fight the disease by inoculating pus from ripe plague buboes.

Samoilovich was active in many plague epidemics. Catching the infection during one of the last epidemics he had the disease in a light form and soon recovered. He explained this by the fact that he had been in the habit of rubbing pus from buboes during operations into cuts in his fingers, thus performing inoculations which later saved him.

Samoilovich's approach to the study of the "invisible world" was subtler and more scientific than that of his predecessors and contemporaries. Forestalling the discovery of the plague organism by 120 years (it was discovered in 1894), he fearlessly fought this dreaded scourge of mankind and is rightly considered to be one of the founders of microbiology.

Further developments in microscopy and in the techniques of laboratory research yielded fresh discoveries early in the 19th century. Bassi discovered the microscopic fungus which causes muscardine, the disease of silkworms, and Schönlein identified another fungus, the pathogen of mange.

During a microscopic examination in 1849 of the blood of animals affected with anthrax, Pollender observed rod-shaped and filiform bodies; he published this discovery but did not attach much importance to it. The following year Davaine and Rayer, laboratory workers of Louis Pasteur, discovered the same bacilli in the blood of cows

affected with anthrax and Davaine thought they were the pathogens of the disease. Between 1855 and 1857 Brauel (in Russia) established that the blood of people and animals stricken with anthrax is contagious; he, too, observed in their blood the bacilli which Pollender had discovered.

In 1873 Otto Obermeier discovered a spirochaete in the blood of persons ill of recurrent fever, but like Pollender, he did not attach much importance to his discovery.

* * *

It was Leeuwenhoek who first noticed some "globules" in beer wort and nearly 200 years later, in 1834, Cagniar-Latour, Schwann and Kützing expressed the idea that these "globules" were the cause of fermentation in beer. This, however, was not more than a surmise and the scientists offered no proof of it.

At that time fermentation was believed to be a purely chemical process, in accordance with the theory propounded by Liebich and Bercelius, two well-known chemists of the 19th century. This theory, shared by the majority of contemporary scientists, held that microorganisms had nothing to do with the process of fermentation.

The famous French scientist Louis Pasteur was a chemist, too, but being specially interested in fermentation he began to study its causes. While examining sour milk under the microscope Pasteur discovered in it small "globules" and short rods. The "globules" were smaller than those found in beer. Observations showed that the "globules" and bacilli in sour milk grew and their number rapidly increased, and therefore he concluded that they must multiply. Pasteur added minute quantities of sour milk containing bacilli to fresh milk, thereby causing lactic acid fermentation.

The subsequent study of alcoholic fermentation showed Pasteur that the production of alcohol and carbon

dioxide from sugar is the result of the activity of live "globules"—yeast cells. He thus established that fermentation is caused by microbes.

The publication of Pasteur's works on fermentation excited keen interest in all countries, particularly in Russia, Britain and Denmark.

In 1874 O. Baranetsky, a Russian chemist, criticized the chemical theories of fermentation of Liebich, Bercelius and other chemists and upheld Pasteur's view. In later years Pasteur's researches into fermentation were continued by D. Ivanovsky and S. Kostychev.

* * *

A letter addressed to Pasteur by an Englishman explained why there was no increase in the demand for French wines after the signing of a trade agreement, a fact which greatly bewildered France. The reason was that English wine-merchants suffered grave losses on account of the wines being affected with disease.

Pasteur examined under the microscope different wines, both unaffected and diseased. He discovered that wine diseases were caused by various microbes. He then sought for ways of protecting wine from the harmful micro-organisms. After a multitude of unsuccessful experiments he found a simple method: heating. In wines which were heated to 140°F. for 30 minutes the microbes were killed. This method prevented wine from spoiling and saved French wine-makers from great losses.

Pasteur studied fermentation in beer wort and found that fermentation in beer was caused by certain yeast fungi. He also discovered that diseases in beer were caused by various micro-organisms. To preserve beer, Pasteur proposed heating it, just like wine; heat-treatment at 131° to 140°F. kills microbes and prevents beer from spoiling.

The method proposed by Pasteur was named after him—pasteurization. Today it is widely used not only in

wine- and beer-production but also for preventing the development of injurious and pathogenic microbes in milk and other perishable products.

* * *

Pasteur discovered the method of combating the diseases of silkworms. In the 19th century silkworm-breeding had spread from Asia to all European countries where the mulberry-tree, the food of the silkworm, could grow. One of these countries was France.

In 1847-48 silkworms in France were affected with a disease which received the name of pebrine (from *pebre* which in the Languedoc dialect means a pear, because the spots on affected silkworms resembled the seeds of pear). Flacherie was another disease of the silkworm. Before many years had passed the diseases spread to all European and Asiatic silk-producing countries and by 1864 healthy silkworm eggs were only available in Japan. All the measures taken by the breeders were of no avail.

In 1865 the government and manufacturers of France appealed for assistance to all leading scientists, including Pasteur.

After numerous investigations Pasteur alone succeeded in proving that both pebrine and flacherie were infectious diseases produced by specific bacilli. Pebrine is caused by myxosporidian organisms and flacherie, by a filtrable virus.

At that time infectious diseases, as well as many other phenomena, were explained by fermentation and that is why Pasteur's discoveries attracted so much attention among scientists.

Pasteur's discoveries regarding lactic acid fermentation led Lister, a well-known English surgeon, to a very important conclusion. Since fermentation results from the activity of micro-organisms, he reasoned, all gangrenous complications setting in after injury or

surgical operations must be due to minute living organisms entering wounds and causing blood-poisoning. In Pasteur's time very simple operations often involved blood-poisoning and death.

This was especially true in time of war.

Sédillot, a learned French surgeon, made the following observation during the Franco-Prussian war of 1870: "Places where wounded are massed are easily recognized by the strong putrid odour. Hundreds of thousands of pale wounded men, their faces still alight with hope and a will to live, perish of hospital gangrene between the eighth and sixteenth day after injury."*

Acting on Pasteur's theory Lister introduced in 1867 a new method of combating death from operations and wounds. He sprayed with a pulverizer a solution of carbolic acid around the wounded, thus killing microorganisms in the air. The surgical instruments and dressings, too, were treated with carbolic acid. This method, which Lister called antiseptics,** although much inferior to the antiseptical and aseptical methods used today, greatly reduced blood-poisoning and mortality from wounds and operations.

Several years before Lister, N. Pirogov, an outstanding Russian surgeon, had established that pyaemia, the

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* Gas gangrene (or hospital gangrene as it was then called) is caused by anaerobic bacteria (see Chapter II) and usually is a complication of wounds inflicted by mines or other artillery missiles and contaminated with earth. Gangrene causes necrosis of tissues around the wound and the poisoning of the organism with bacterial toxins.

** *Sepsis* is invasion of the blood by streptococci, staphylococci or other microbes.

Antiseptics is the chemical protection of the organism from the development of microbes in it, i.e., disinfection, as for instance, with carbolic acid, lysol, etc.

Asepsis embraces the physical methods of protecting the organism from infectious microbes such as the sterilization of surgical instruments, dressings, linen, etc., through boiling, pressurized steam treatment at 248° F., dry heat at 320° F., etc.

scourge of contemporary hospitals, was a fermentative process, involving the action of bacteria. When Lister's article "The Antiseptic Principle in Practical Surgery" appeared in 1867, Pirogov stated that "the success of the antiseptic treatment of wounds and Lister dressings has fully borne out my teaching."

* * *

While investigating the problem of fermentation Pasteur often mused over the causes of infectious diseases, particularly anthrax, a fatal cattle disease which annually destroyed 20 per cent of sheep in France, and on some farms 30 per cent and 50 per cent of the flocks.

The study of the disease enabled Pasteur to identify the anthrax bacillus and to cultivate it in meat broth, in the humour of bull's eye, and in other culture media. Experiments convinced Pasteur that these bacilli (or bacteridia, the term suggested by Davaine, which Pasteur retained) are the causative organisms of anthrax. But that did not satisfy Pasteur: he wanted to find a means of defence against anthrax.

The following incident took place when Pasteur was studying the organism of chicken cholera, which he bred in broth. Pasteur and his assistants interrupted their laboratory work for summer holidays, but a culture of the cholera bacillus was left by chance in an incubator and remained there for a few weeks. When they came back to work they used this old culture to inoculate chickens; the fowls contracted the disease but did not die of it. Once more they were infected with a young and vigorous culture, but still they survived the disease while other chickens infected with the same culture died in due time. Pasteur concluded that the virulence of the culture kept for several weeks in unfavourable conditions had been so reduced that the disease had taken a mild course and rendered the chickens immune.

Pasteur knew that Jenner, an English physician, had immunized human beings against smallpox by inoculating them with the weak cowpox.* But Jenner had not found the causative organism of smallpox and did not know why his vaccinations immunized human beings. The experiment with chicken cholera showed Pasteur why vaccination immunized the human organism against smallpox.

This incident indicated the way to obtaining attenuated microbe cultures for producing immunity.

The efforts of Pasteur and his pupils, Roux, Chamberland and others, led to the discovery that a culture of the anthrax bacillus cultivated in chicken broth from 12 to 20 days at a temperature of 107.6°-109.4°F. becomes so weakened that sheep inoculated with it contract the disease in a mild form.

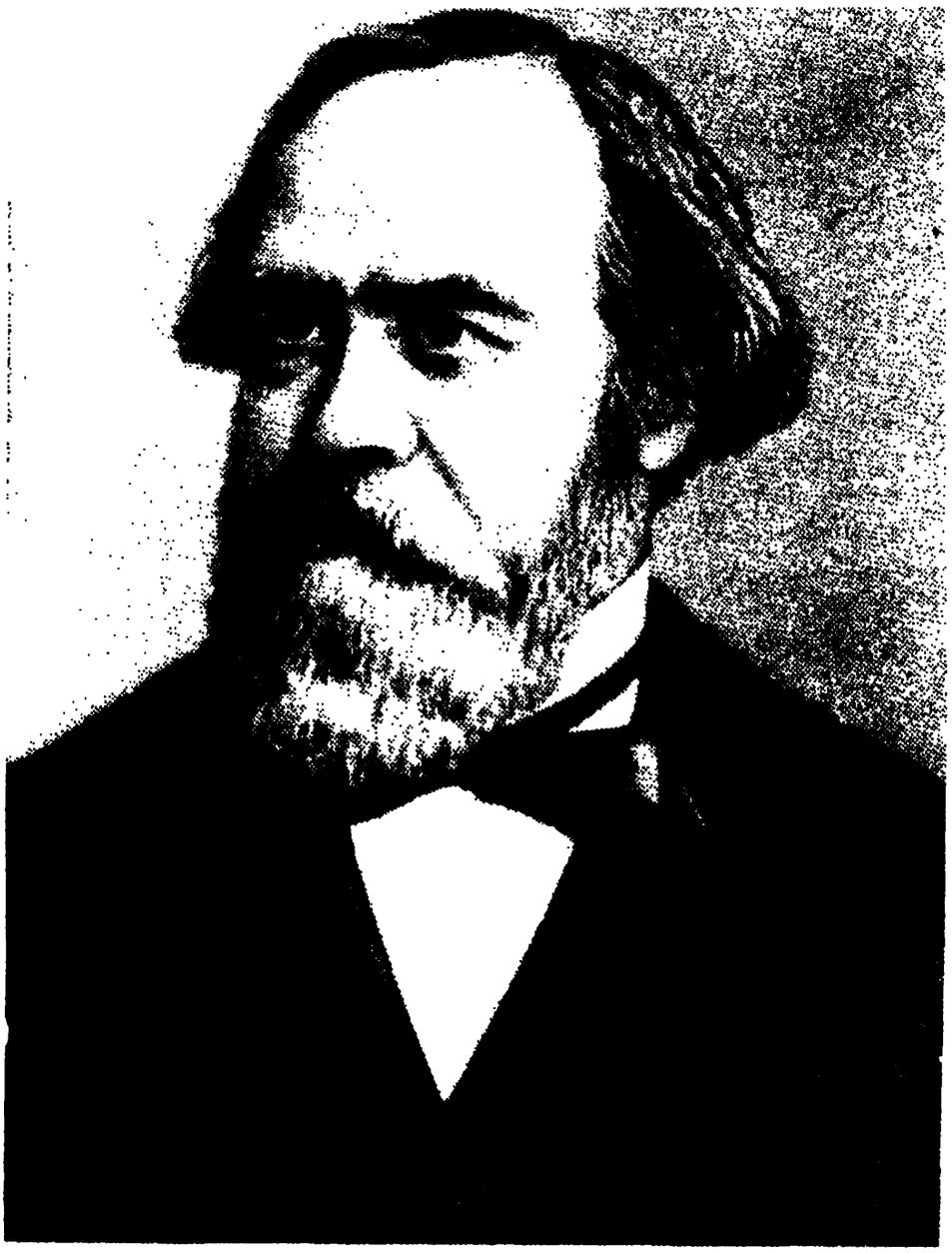
Passages of the anthrax bacillus, i.e., its cultivation in fresh portions of broth, produced bacterial growths with weakened virulence. This greatly facilitated and made less expensive the preparation of vaccines as Pasteur called this method in honour of Jenner: the cowpox with which the latter had inoculated man was called vaccine from the Latin *vacca*—cow.

On the suggestion of the Agricultural Society in Melun Pasteur's vaccine was given a public test.

Twenty-five rams and six cows were twice inoculated with the vaccine on May 5 and 17, 1881. A few days after the second vaccination the animals, as well as 29 control stock (25 rams and 4 cows which had not been previously vaccinated), were inoculated with a lethal dose of anthrax bacillus culture.

When, on June 2, 1881, Pasteur arrived at the farm where the experiment was being carried out, he was greeted by a storm of applause from the assembled delegates of the Agricultural Society, veterinaries, doc-

* For Jenner and vaccination see Chapter XI.



Lev Tsenkovsky

tors, and others. The carcasses of 22 rams lay dead on the ground, beside them 2 rams were dying, while the last of the 25 unvaccinated rams showed all the characteristic symptoms of anthrax. All the vaccinated animals were in excellent health.

Thus the problem of combating anthrax was solved and mankind received a reliable weapon against this devastating disease.

In Russia, too, animal husbandry suffered from anthrax, just as in other European countries. The Free Economic Society whose aim was to find means of

protection against anthrax commissioned Prof. Lev Tsenkovsky, a botanist and founder of the Russian school of bacteriology, to Paris to master under Pasteur his method of preparing the anthrax vaccines. He went in company with Prof. A. Rayevsky, agronomist P. Kostychev, and several other specialists. But to their astonishment Pasteur told them that his laboratory was meant for research and not for instruction. He showed them round his laboratory, outlined the general principles of preparing the vaccines and allowed them to observe the vaccination of a large number of sheep with the anthrax vaccine. That was all the "instruction" the Russian scientists got from Pasteur.

His behaviour may have been due to the fact that he had sold his vaccine rights to a joint-stock company set up specially for that purpose. Another probable reason was that Pasteur carried on all his researches in two small laboratories of the Ecole Normale, where for 30 years he lived in constant fear for his discoveries, in constant strife with numerous opponents, and where, perhaps, there was no room for instruction.

That was probably why Pasteur would not admit any foreigners or even his own countrymen to his laboratory.

Tsenkovsky had to study the pathogen of anthrax independently and to find his own method of preparing live anthrax vaccines.*

Tsenkovsky worked out methods of preparing vaccines that were better than Pasteur's. From then on until quite recently Tsenkovsky's vaccines were successfully used in Russia and the Soviet Union.

A few years ago Soviet scientists developed a new anthrax vaccine which is far superior to both Pasteur's and Tsenkovsky's vaccines.

* * *

* A. Metelkin, *L. Tsenkovsky, Founder of Russian School of Microbiology*, Medgiz Publishing House, 1950.

Everyone knows what "hydrophobia" and "rabies" mean, but not many have a clear idea of this fatal disease. Indeed, few people except specialists have had the occasion to observe this disease in animals, and still less in man. Even doctors know it mostly from books and manuals. But in the old days hydrophobia occurred frequently enough and in France alone 770 persons died of it in between 1850 and 1876.

Physicians of ancient times tirelessly sought effective cures against this disease. But only cauterization of the bitten part, proposed by Celsus in the first century A.D., sometimes yielded positive results. All other methods of treatment suggested in the subsequent 17 centuries were mere inventions, which had nothing to do with science.

All efforts to discover the cause of hydrophobia were also in vain. Galletier succeeded so far as to establish that the hydrophobia virus affected the nervous system. Another thing known for certain was that saliva of rabid beings was infectious. Such was the state of things when Pasteur turned his attention to the problem.

His first step was to look for the virus, for Pasteur felt convinced from the very beginning that the disease was caused by some microbe. He experimented on rabid dogs brought to him from a special hospital for dogs. As a result of experiments which might have cost him his life, Pasteur established that the blood of rabid subjects was not infectious, but that their saliva was undoubtedly so; also, that the disease affected mainly the nervous system.

Here is a description of the disease by Emile Roux, one of Pasteur's closest associates.

"When observing the manifestations of rabies one clearly sees the virus spreading along the dog's nervous system. Agitation and paroxysms of frenzy indicating the excitation of the cortex are followed by a change of the voice and difficulties in swallowing. This means that the medulla oblongata and the nerves originating from

it are affected. Finally the brain itself becomes affected and the whole pathologic process ends in paralysis."

The period of incubation lasts as long as it takes the virus to make its way from the bitten part to the central nervous system.

Since the brain is the seat of the virus, the disease of a healthy animal can be best caused by introducing the virus directly into the brain. After a long series of preparatory experiments Pasteur and Roux introduced the rabies virus into the brains of dogs.

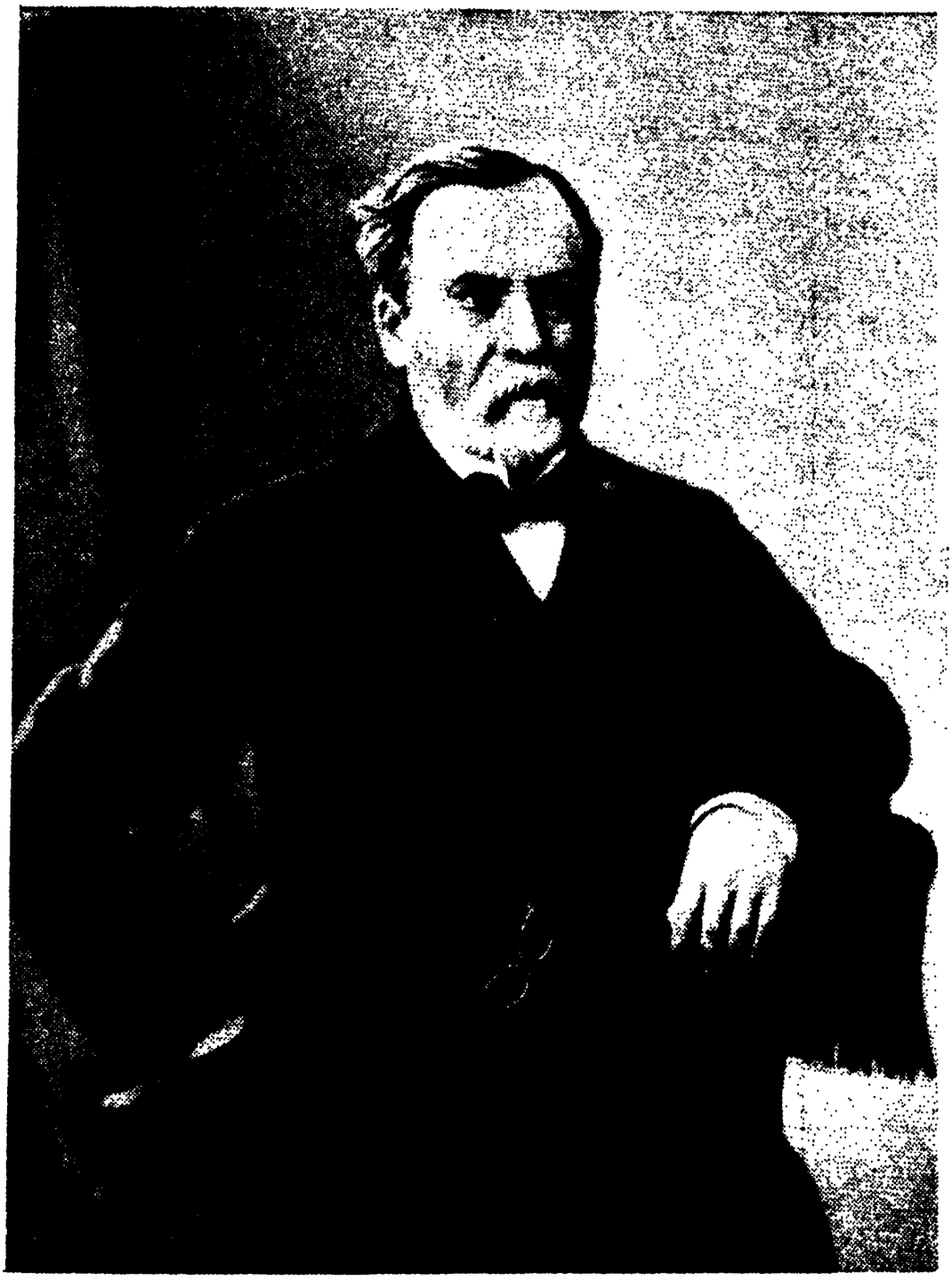
For two weeks all dogs infected through the brain seemed quite well, but after fourteen days the symptoms of rabies manifested themselves quite clearly and the dogs died of paralysis.

Pasteur knew from the works of the French scientist Galletier that rabbits were especially susceptible to rabies, and after successful experiments on dogs he passed on to rabbits.

By inoculating the virus directly into the brain of a rabbit Pasteur obtained the disease in the animal on the 15th day. Another rabbit was then infected with the virus by introducing a piece of the first rabbit's brain into its brain. The second rabbit contracted the disease after a shorter period of incubation. A third rabbit was infected in the same manner from the second rabbit which had died of the disease. In this instance the incubation period was still shorter.

Pasteur went on infecting healthy rabbits with the brains of those that had perished until the incubation period was reduced from 14 to 7 days. The subsequent infections did not bring about any further reduction of the incubation period. The virus that produced the 7-day incubation period was called fixed.

Pasteur's next step was to prepare a serum for antirabic inoculations; he wanted to prepare a virus so weakened that it would be incapable of causing the disease but would produce a lasting immunity. This was the



Louis Pasteur

most difficult task, since to solve it, the virulence of the brain of a rabbit that had died of the fixed virus had to be weakened to the necessary degree.

And Pasteur solved it. He dried the spinal cord of rabid rabbits, and the virulence of the cord gradually diminished in the process of drying until it was completely lost on the 14th day. Healthy dogs were inoculated with this harmless cord. Next day the dogs received

a dose of cord dried for 13 days, the following day one that had been dried for 12 days, and so on. The drying time was gradually reduced with a corresponding increase of virulence, which finally was so strong that dogs unprepared by previous treatment contracted the fatal disease.

The effectiveness of the inoculation was checked in the two following ways: some of the inoculated dogs were infected with fresh virulent virus, which had no effect on them; others were put into a cage with healthy dogs that had not been inoculated previously, and then a rabid dog was introduced into the cage. Only the non-inoculated dogs died of bites of this dog.

Although Pasteur's experiments proved the effectiveness of his inoculation he did not as yet dare to try it on human beings and continued experimenting on animals.

Life itself forced Pasteur to start treating man of this disease and to find the best methods of inoculation.

Early in July 1885, a little boy was bitten by a mad dog in a village near Villay in Alsace. The following day the boy's mother brought him to Pasteur. The boy, bitten fourteen times, writhed in pain.

Pasteur decided to take the chance and gave the boy the same treatment he had used on dogs.

How can one describe Pasteur's anxiety during the ensuing month! But the treatment proved successful: the wounds healed, the boy recovered and played gaily in the yard before Pasteur's laboratory.

A second case which occurred the same summer settled the problem of the anti-rabic vaccination of human beings.

Jupile, a shepherd, was sent to Pasteur from the Jura Department. The boy had fearlessly beaten off a mad dog that had attacked his chums but got bitten himself. When after treatment similar to that administered to the first boy Jupile did not contract the disease, Pasteur

realized that he not only had a weapon against hydrophobia, but that he knew how to use it.

When Pasteur published the results of his successes he had people bitten by mad dogs coming to him from all parts of France.

The news of Pasteur's discoveries spread all over the world. When a mad dog bit four poor children in New York, a public fund was raised and the youngsters were sent to Pasteur. They received a course of treatment and returned home in the best of health.

* * *

Russian scientists and physicians were quick to appreciate the importance of Pasteur's discovery. Nikolai Gamaleya, a young Odessa doctor, was commissioned to Paris in February 1886 He quickly mastered the methods of preparing the vaccine and its application.

On June 23, 1886, the first Pasteur Station outside of France was founded in Odessa. Before the year was out nearly 400 persons bitten by rabid dogs had been successfully vaccinated there. That same year Pasteur stations were opened in Warsaw, Samara, Petersburg and Moscow.

But Pasteur's discovery of a potent weapon against hydrophobia did not only arouse enthusiasm. There were also opponents who could not or would not believe in the effectiveness of his method.

The few cases when patients died in spite of the treatment, because vaccination was applied too late to be of any use, added weight to the arguments of Pasteur's enemies who demanded that his inoculations be stopped.

These sentiments found their way to the press. One newspaper wrote:

"A person who steals silver spoons is sentenced to hard labour while Pasteur who murders people enjoys freedom."

Nikolai Gamaleya, who, in later years, became an outstanding scientist and member of the Academy of Science, was beginning his researches at the time. His observations in Paris and at the Pasteur Station in Odessa revealed why some of the inoculations performed at Pasteur's laboratory were unsuccessful. The reason was that the treatment was applied too late when the virus had penetrated to the central nervous system and when no cure could save the patients.

In speaking of Pasteur we could not go into all his discoveries in microbiology; we have touched only on the most important of them, landmarks not merely in microbiology but in the history of humanity.

A pupil of Pasteur's has given the following graphic description of the work of his teacher: "After the primitive man had ceased to fear wild beasts the history of civilization knows of no more decisive step than that made by Pasteur who has taught man to fight still more dangerous ubiquitous microbes."

Pasteur's greatness lies not in his genius alone: he always strove to apply his knowledge to save humanity from fatal diseases, and always opposed war by word and deed. During the Franco-Prussian war of 1870 Pasteur expressed his protest against German aggression by returning to Bonn his Honorary Doctor's Diploma which Germany had awarded to him for his services to science.

* * *

Robert Koch, a contemporary of Pasteur, but 21 years his junior, applied Pasteur's method of cultivating bacteria in broth and other culture media. Once while working in his laboratory Koch chanced to look at a piece of boiled potato which had been left on the table for two or three days. His interest was aroused by the slightly protruding spots on the potato which were of red, yellow, orange, whitish and other colours. Koch examined them with a microscope and discovered that some spots were col-

lections of minute rods, others of globules. He decided that different bacteria had settled on the potato from the air and their growths produced the varicoloured spots or droplets.

This gave Koch an idea to breed bacteria on potato or some other solid medium. He began adding gelatine

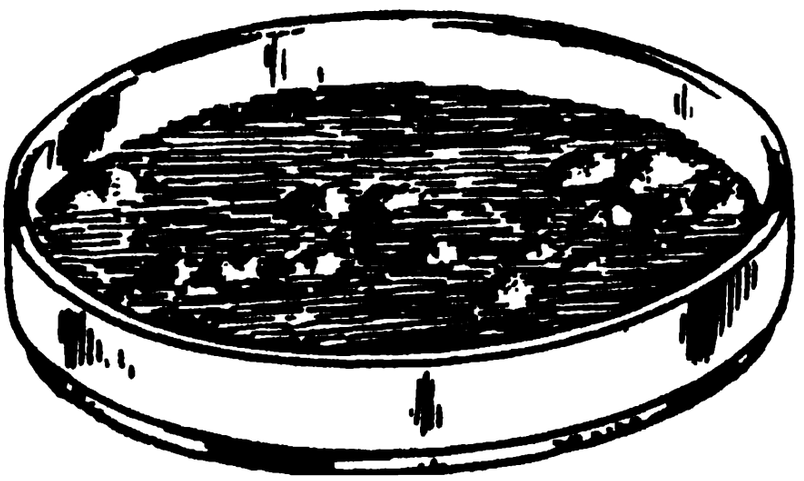


Fig. 8 Colonies of bacteria bred on a solid culture medium

to broth which he poured out on glass plates where it solidified. On the surface of such a medium it was easy to breed colonies of different bacteria, each colony consisting of strictly uniform organisms (Fig. 8).

In addition Koch used a new method of microscope preparations suggested by L. Geidenreich, a Russian scientist. Bacteria were put on the stage of a microscope, killed and fixed by heating over the flame of a spirit-lamp. Then aniline dyes were applied to the preparation, which presented a vivid picture under the microscope.

It was found that different microbes acquired different colours from the same dyes; this greatly facilitated their identification.

Advanced laboratory techniques and improved microscopes helped Koch to make several important discoveries. His first objective was to identify the bacillus of tuberculosis. This involved long and assiduous work; Koch

tried various methods of staining bacteria and experimentally infected with tuberculosis a large number of guinea-pigs. Finally, while examining under the microscope the lungs of a person who had died of galloping consumption he discovered in them thin, slightly curved bacilli which congregated in places to form clusters. The guinea-pigs infected by material from these lungs contracted tuberculosis and perished. The same bacilli were discovered in the preparations of the lungs of the dead guinea-pigs.

The tubercle bacillus is often called Koch's bacillus, after its discoverer.

Several years later Koch prepared tuberculin which he intended to use as a remedy against tuberculosis. Unfortunately, tuberculin proved to be but a means of diagnosing tuberculosis in cases when the prevailing conventional methods were of no avail. Recently, however, a modified and weakened form of tuberculin has been accepted for treating tubercular patients. The therapeutic dosage of this "new" tuberculin depends on the condition of the patient, the character of the process, the blood picture and the patient's susceptibility to tuberculin.

In 1883 Koch was sent to Egypt where there was an epidemic of Asiatic cholera. Koch's study of the aetiology of the disease resulted in his identifying the specific organism of cholera. To verify his discovery, Koch went to India where cholera was always present. After a great number of investigations of cholera patients and corpses Koch became convinced that the comma bacillus was the pathogen of cholera.

Koch, one of the founders of modern bacteriology, has made valuable contributions to the science.

* * *

Ilya Mechnikov, an outstanding Russian scientist whose early works on zoology and embryology brought him world recognition, is well known. His chief service

Robert Koch

to science is, however, the theory of phagocytosis, the foundation of the teaching of immunity, which he had created.

This is what Mechnikov himself said of this theory he had evolved when living in Messina* (1883). "One evening when the rest of the family had gone to the circus to see some wonderful monkeys and I was examin-

* At that time Mechnikov was studying the comparative evolution of the invertebrata which abound in the Mediterranean. He lived first on the shores of the Bay of Naples and later in Messina.

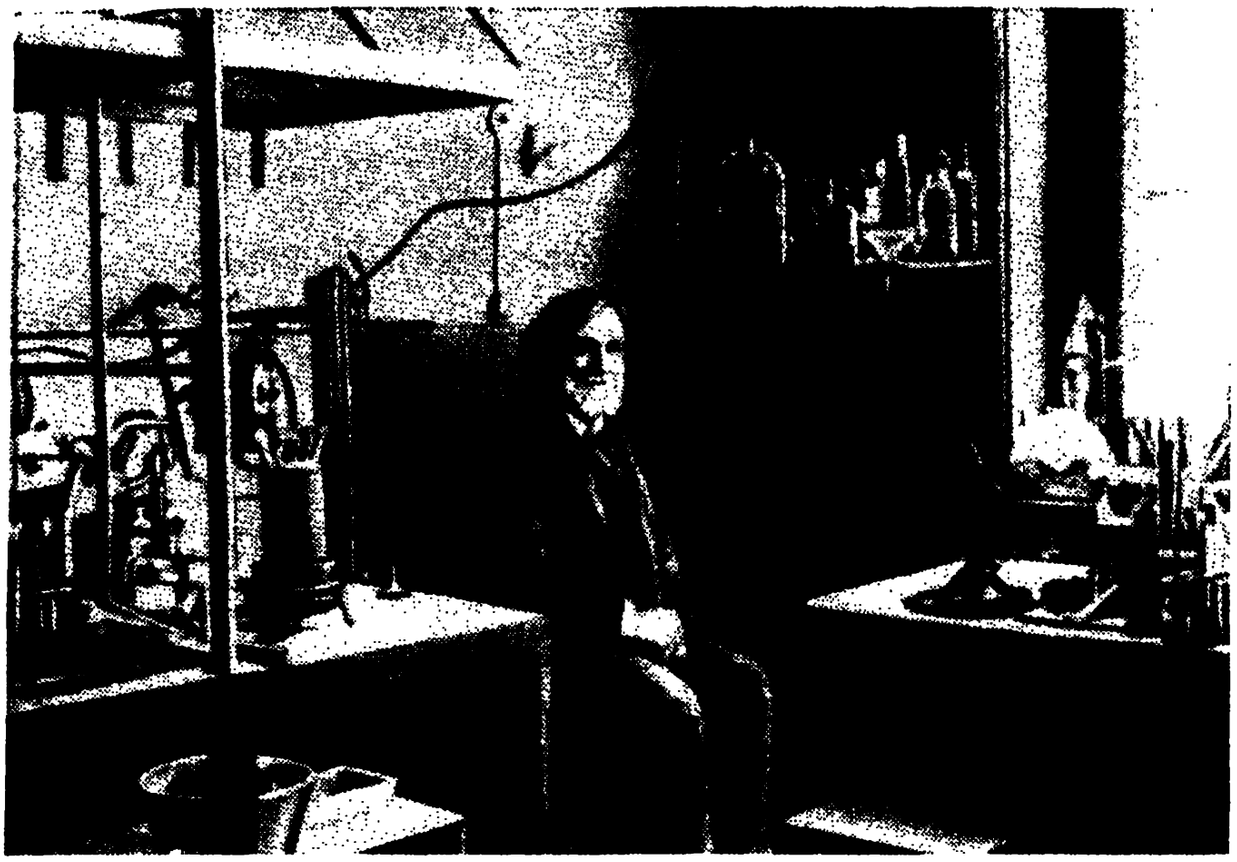
ing mobile cells of the transparent starfish larva, it suddenly occurred to me that such cells must function as defensive cells protecting the organism against harmful agencies. I was so excited by the idea and its implications that first I walked about the room and then went out to the seashore to think the matter over. I told myself that if my conjecture was right a splinter thrust into the body of the starfish, which has neither a vascular nor a nervous system, must shortly become surrounded by wandering cells, just as in the case of a splinter in a man's finger. No sooner said than done. I picked some thorns from a rose-bush in the tiny front garden where a few days before a tangerine-tree had been used as Christmas-tree to entertain the kiddies. I thrust the thorns under the skin of the beautiful crystal-clear starfish larvae. Of course, I was restless all night long, and early in the morning saw that my experiment was successful. This experiment laid the foundation of the theory of phagocytosis to which I had devoted 25 years of my life."*

In observing unicellular protozoa, as for instance amoeba, we can see it thrust out protoplasmic *pseudopodia* engulfing minute particles of food, which are then assimilated within its body.

The body of lower animals, such as the ciliary worms which have no alimentary canal, is an agglomeration of a great number of cells. When food enters the body cavity through the mouth, the wandering cells (called the mesodermal cells) envelop the food and digest it; this process is known as intracellular digestion and was discovered by Mechnikov. He named these wandering cells phagocytes from the Greek *phago*—to devour and *kytos*—a cell.

While studying this problem Mechnikov made up his mind to see the reaction of wandering cells to pathogenic

* I. Mechnikov, *Recollections*, U.S.S.R. Academy of Science Publishing House, 1946, pp. 74-75.



Ilya Mechnikov

microbes. He chose for this purpose a small crustaceous—the daphnia. Its transparency enabled Mechnikov to see what occurred inside its body. Sometimes he saw needle-like spores of *Monosporia bicuspidata*, a parasitic fungus, getting into it along with food. The spores had pierced the daphnia's alimentary canal and penetrated into its body. Here they were attacked by the wandering cells, which devoured and digested the intruders. When, however, the spores were too numerous for the wandering cells to devour, fungi developed from the spores, which filled the daphnia and finally caused its death.

These observations showed that the mobile cells of an organism are its weapon against parasites. From this Mechnikov drew the conclusion that the organisms of higher animals and of man, too, fight harmful microbes with the aid of special cells called phagocytes.

Mechnikov turned his attention to the mobile blood cells—the leucocytes.

Prior to Mechnikov's discovery scientists in many

countries held that inflammation was a disease of the walls of the blood-vessels and that leucocytes constituted a favourable medium for the development of pathogens.

Mechnikov proved that inflammation was "the organism's reaction to the influence of various pathogenic agencies, chiefly pathogenic microbes."* If, for instance, a man had a splinter in his finger and if pyogenic microbes had penetrated into the wound along with the splinter, an inflammation would soon set in; the leucocytes collected around the splinter would seize the microbes, kill and devour them.

Mechnikov emphasized the protective function of inflammation and clearly stated that the most important aspect of the inflammatory process was the aggregation of phagocytes and their attacking of microbes and other irritants (Fig. 9). Mechnikov discovered that the spleen, the marrow, the lymphatic glands and some other organs of the vertebrata contain special cells which he named macrophages and which also perform a protective function.

All the experiments and observations made by Mechnikov invariably confirmed his hypothesis. In cases when intensive phagocytosis was observed the disease did not develop and the animal either remained healthy or survived the disease and recovered. But if there were no phagocytosis, the animal contracted a severe form of the disease after infection, often with fatal results.

But sometimes—and the opponents of the phagocytosis theory have pointed this out—pathogenic bacteria, as for instance gonococci, are not killed and digested by the leucocytes. Mechnikov considered this natural, for "we must admit the existence of different forms and stages of adaptation on the part of both phagocytes and bacteria. . . ." It is possible, therefore, that "in due time some

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* I. Mechnikov, *An Essay on the Present State of the Problem of Inflammation*, Medgiz Publishing House, 1946.

bacteria are able to withstand the digestive action of the phagocytes, and instead of being their food become their parasites."

There were many who tried to disprove Mechnikov's theory. One of their strongest arguments was the humoral theory, which held that it was antibodies formed in the lymph and the humours of different organs, and not the leucocytes and macrophages which protected the organism from pathogenic microbes and their toxins.

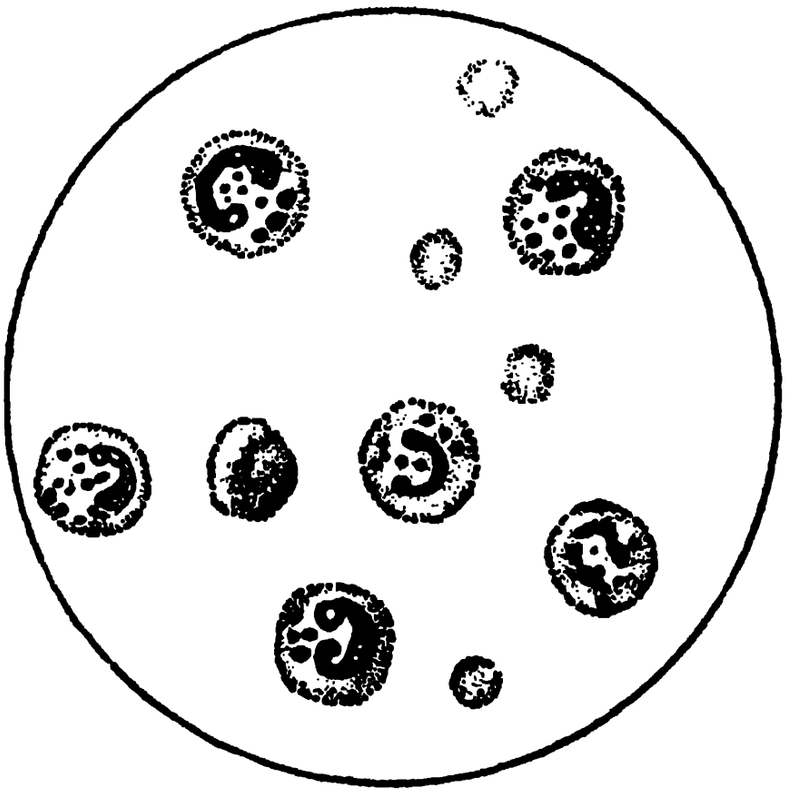

Fig. 9 The phagocytosis staphylococci

V. Isayev, a Russian scientist who worked in the laboratory of Pfeiffer in Germany, made the following experiment. He inoculated a guinea-pig with the cholera vaccine and then injected into its peritoneum an extremely virulent culture of the cholera vibrio. Extracting the peritoneal fluid Isayev saw that the vibrios had been killed, part of them having disintegrated into minute grains. There were almost no leucocytes in the fluid. This experiment convinced Pfeiffer that the leucocytes—the phagocytes—had taken no part in protecting the organism of the guinea-pig from the vibrio and that the organism formed antibodies which killed pathogenic microbes.

Mechnikov did not try to refute the humoral theory until, by a series of most brilliant and clever experiments,

he could prove that the antibodies formed in the lymph and the humours of the organs are intimately connected with the phagocytes. Such antibodies as antitoxins, for instance, are produced at the decomposition (lysis) of the phagocytes. If the phagocytes do not decompose (dissolve), no antitoxins are formed and they attack the microbes directly and devour them.

Mechnikov's fight against the opponents of the theory of phagocytosis went on for almost twenty-five years. His theory won world recognition and is at present being confirmed by new researches of Soviet and foreign scientists.

The phagocytosis theory of immunity is the pride of Russian science and is rightly considered to be one of the greatest achievements of biology and medicine.

Unable to do any fruitful research in tsarist Russia Mechnikov went to Paris where he spent the last twenty-eight years of his life working at the Institut Pasteur which he had helped to found. Living abroad Mechnikov maintained close contact with his home country. Some of the leading Russian bacteriologists of the beginning of the 20th century—L. Tarasevich, A. Bezredka, I. Savchenko, P. Tsiklinskaya—were Mechnikov's pupils.

Mechnikov enjoyed the respect of Pasteur and his pupils. After Pasteur's death Mechnikov headed the Institute together with Emile Roux, his friend and an associate of Pasteur's. The Soviet people honour Mechnikov whose life was devoted to the good of humanity and the triumph of science. The role of microbes in Nature and in the life of man was not known until Pasteur and Mechnikov made their discoveries. After this microbiology began developing at a rapid pace.

The last two decades of the 19th and the first decade of the 20th century were years of new discoveries in microbiology, including the discovery of organisms of leprosy, malaria, diphtheria, typhoid fever, glanders, tetanus, brucellosis, botulism, plague, and of other infectious diseases.

Russian scientists made substantial contributions to this rapid succession of discoveries.

Mention has already been made of the effective anthrax vaccine developed by Tsenkovsky. G. Minch, who identified the spores of anthrax in 1873, performed a heroic deed by infecting himself with the blood of a recurrent fever patient, proving thereby that the causative organism—the spirochaete—is contained in the blood. But that was not all. Minch's most important discovery was that recurrent fever is transmitted not from man to man but through blood-sucking insects, i.e., that some infectious diseases are transmitted through insects.

O. Mochutkovsky, another Russian scientist, infected himself with typhus by injecting the blood of a typhus patient, thus proving that the causative organism of typhus has its seat in the blood.

In 1875 F. Lesh identified the causative organism (*Entamoeba histolytica*) of amoebic dysentery, which was once again discovered in 1903 by Schaudinn.

L. Geidenreich, an outstanding bacteriologist of his day, proposed the use of Papin's digester as the autoclave for sterilizing culture media (broth and other media), which Koch would not use. In 1883 he invented shallow glass vessels for breeding microbes on solid media; the flat vessels with flat lids are to this day used in microbiological laboratories (Fig. 10). Later the same vessels were invented by Petri, a pupil of Koch's, and as is often the case, the real inventor was forgotten and the vessel became known as the Petri dish.

In 1898 P. Borovsky, an interne of the Tashkent Military Hospital, discovered the organism of a disease usually affecting the skin and, most often, the face. It turned out that the organism was not a bacterium as had been previously supposed, but a protozoan. Five years later this same disease was described by Leishman, a British scientist, and by Donovan, but in the form affecting internal organs. Borovsky, a modest and unassuming man,

did not insist on his priority; he was forgotten and the disease was named leishmaniosis, after the second discoverer of the pathogen.

A. Polotebnov and B. Manasein noticed that the growth of microbes was retarded by a certain green mould—penicillium. Some 70 years later Oxford scientists discovered a method of extracting from some varieties of the penicillium fungus penicillin, a potent remedy against many infectious diseases.

It will be recalled that the idea of utilizing the antagonism of microbes belongs to Mechnikov who suggested drinking curdled milk which contains a multitude of *Bacilli acidi lactici*, exercising a depressing influence on the bacterial flora in the intestines. This problem as well as that of immunity will be treated in greater detail in Chapter XI, here we shall mention some other branches of microbiology which owe their development to Russian scientists.

Further development of microbiology showed that the complex and vital processes occurring in cultivated soils are the work of the myriads of micro-organisms abounding in them. The secrets of many of these invisible organisms, the influence they exercise on plant growth and on soil productivity have been disclosed by S. Vinogradsky, D. Ivanovsky, V. Dokuchayev, P. Kostychev, V. Omelyansky, V. Butkevich, N. Khudyakov, M. Voronin, B. Isachenko, E. Mishustin, and N. Krasilnikov. Rus-

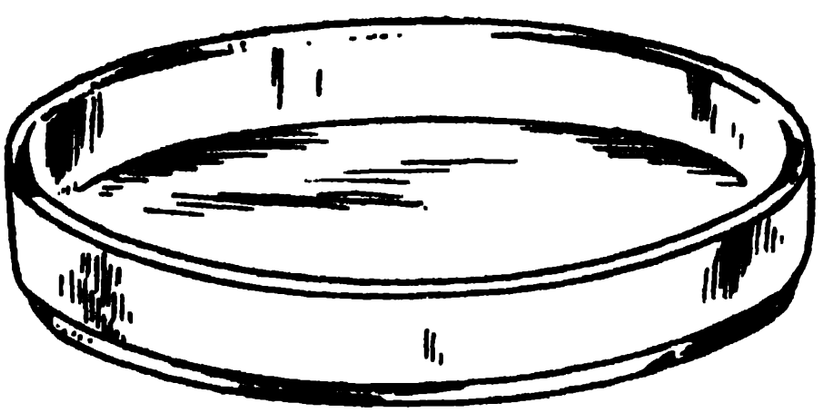

Fig. 10 The Geidenreich-Petri dish

Sergei Vinogradsky

sian scientists established also the role of different microorganisms in the processing of agricultural products.

In 1889, at the age of 33, S. Vinogradsky, already well known for his research on the activity of iron and sulphur bacteria, took to studying the activity of microorganisms in soil.

His new and original methods of microbiological research soon revealed the presence in soils of two types of microbes which produce nitrite and nitrate salts (saltpetre) from ammonia. The microbes were named nitrobacteria (see Chapter III). Vinogradsky's important discovery laid the foundation of soil microbiology, and

his methods have since been used in laboratory work in all countries.

Another complex problem that long awaited its solution was: how do plants receive the necessary nitrogen nutrition in places where it is inadequate? Some European scientists, as for instance Berthelot, believed that microbes might have something to do with it, but this was a mere guess. It was Vinogradsky who furnished the solution. He discovered a microbe which assimilated nitrogen, so essential for plant life, directly from the air. This micro-organism will be dealt with in Chapter III.

Vinogradsky lived to nearly a hundred (born in 1856, he died in 1953) but his old age did not prevent him from working in the field of soil microbiology and from publishing his works which have become the guide for scientists all over the world.

A great contribution was made by Academician M. Voronin who discovered that the nodules of leguminous plants contain bacteria which also assimilate atmospheric nitrogen.

Academician V. Omelyansky, a pupil of Vinogradsky, discovered two types of microbes decomposing cellulose and producing methane and hydrogen. This discovery has largely stimulated the development of industrial microbiology and the science treating of the geological action of microbes. His *Fundamentals of Microbiology* has appeared in its ninth edition and serves as a manual for all microbiologists.

In 1892, D. Ivanovsky, a Russian botanist, while studying the mosaic disease of tobacco, discovered that the sap of diseased plants can infect healthy plants. Passed through the bacterial filter* the sap preserves its virulence, because the causative organism of the disease can pass through the filter which detains common bacteria.

* Bacterial filter is made of unglazed porcelain with extremely fine pores, heated at 2,192°F.

Dmitry Ivanovsky

This was an outstanding discovery proving the existence of such minute microbes as cannot be seen through an ordinary microscope.

At that time the pathogens of infectious diseases were called viruses. Viruses, similar to those discovered by Ivanovsky, pass through the bacterial filter, therefore all ultra-microscopic microbes, parasites of animal and vegetable cells, are called filtrable viruses.

In this short essay we cannot go into the details of every discovery in the field of microbiology, or even give the names of all the scientists, authors of the different discoveries. We shall mention some of the works only of

Soviet scientists who made valuable contributions to the development of world microbiology.

Academician B. Isachenko is one of the first in the list of our contemporaries. About 45 years ago he began studying the activity of micro-organisms in seas and oceans. His extensive studies have largely contributed to the advancement of science, serving as the foundation of a new branch of microbiology—pelagic microbiology.

Prior to Isachenko's discoveries it was held that in the Arctic seas there were no processes caused by the activity of micro-organisms. Isachenko established, however, that the Arctic seas are peopled with a great variety of microbes, including nitro- and nitrogen-assimilating bacteria, having in the seas a function similar to that in soils.

At a later date he investigated Arctic soils on the Isles of Vize and Isachenko where no man had set foot before him.

Isachenko investigated the waters of the Black, Caspian and Marmora seas, and of the Sea of Azov. He established the participation of certain bacteria in the production of hydrogen sulphide at great depths in the Black Sea. He discovered that the cause of the death of fish in the Sea of Azov was the production of an excessive quantity of hydrogen sulphide by bacteria.

With his assistants Isachenko continued the work of A. Verigo, N. Zelinsky, and E. Brusilovsky in investigating the curative muds in the estuaries of Odessa, in the Tambukan lakes near Pyatigorsk, and in other places. This investigation proved that the muds were the result of bacterial activity.

The problem of the spontaneous heating of grain and of other agricultural produce interested scientists as far back as the time of Catherine II, but it was Isachenko and his co-workers who solved it. They found out that the process was the result of the activity of certain thermophilic micro-organisms.

Soviet microbiologists A. Imshenetsky, N. Krasilnikov,

Boris Isachenko

V. Shaposhnikov, E. Mishustin, and others, are making an extensive study of soil and industrial microbiology, of the activity of micro-organisms in various geological processes, and, particularly, in the formation of petroleum. Soviet scientists have also done some meritorious work in food microbiology.

The discovery of penicillin and other antibiotics has revolutionized the treatment of infectious diseases (see Chapters III and XI) and there is good reason to believe that antibiotics will play an important role in the agriculture of the future.

Chapter II

MICROBES

All bacteria, fungi (yeast, mould and ray fungi), and protozoa, are called microbes. Microbes that can be seen with the ordinary microscope are several microns long. Filter-passing viruses, too, are micro-organisms, but they are so small that they are measured in millimicrons.

Microbes can be observed with microscopes magnifying from 100 to 1,000 and more times.

The small globules seen in Fig. 11 are bacteria named *cocci*. The *cocci* grouped in chains (Fig. 11*a*) are called *streptococci* (from the Greek *streptos*—a chain), those grouped in clusters (Fig. 11*b*), which sometimes are like clusters of grapes, are called *staphylococci* (from the Greek *staphyle*—grapes).

In Fig. 12 we see thick and rather short rods whose ends seem to have been cut as if with a knife. The rods are often grouped in chains of some length, and sometimes in threads. In our figure we see the anthrax bacteria magnified 1,200 times.

The thin rods in Fig. 13, which usually form stacks or hedges, are the causative organism of leprosy.

Today the bacteria of typhoid fever and of dysentery are pretty well known. Under the microscope both look very much alike, just little rods, but they cause two different diseases. The two types of bacteria differ in many other respects. The dysentery bacteria, for instance, when observed with a microscope, seem to sway or mill without

moving from their place; but this motion is not produced by the bacteria themselves which are actually stationary, for they have no organs of locomotion. The typhoid bacteria under the microscope present a picture of a multi-

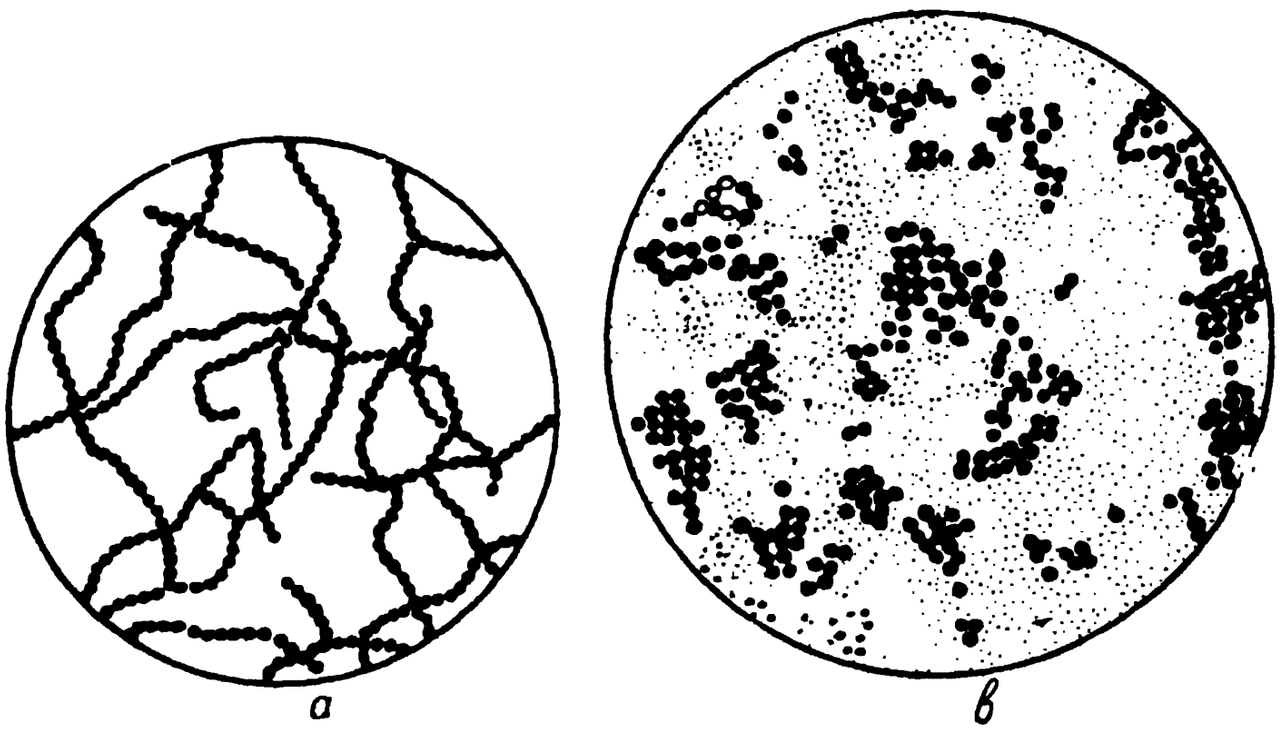

Fig. 11 Streptococci (*a*) and staphylococci (*b*)

tude of minute rods darting swiftly to and fro. These bacteria have thin, thread-like flagella moving like oars and thus carrying them from place to place (Fig. 14).

Leeuwenhoek discovered in dental tartar excessively fine spiral threads. Then Obermeier, too, found microbes resembling these threads in the blood of persons who were ill of recurrent fever. Both bacteria have the same spiral form and are called spirochaetae, but the former cause no infectious disease while the latter are the agents of a severe disease.

A man has fallen ill, he vomits and has diarrhoea. A bacteriological analysis has established the presence of comma-like bacteria in his faeces resembling rice-water. The commas are vibrios. The bacteriologist tests them for some other properties and finally establishes them to be cholera vibrios (Fig. 15).

Numerous and minute tests are essential in this case, for there are many other vibrios, similar in shape but absolutely different in properties. There are, for instance, vibrios which do man no harm while others, outwardly in no way different, cause Asiatic cholera.

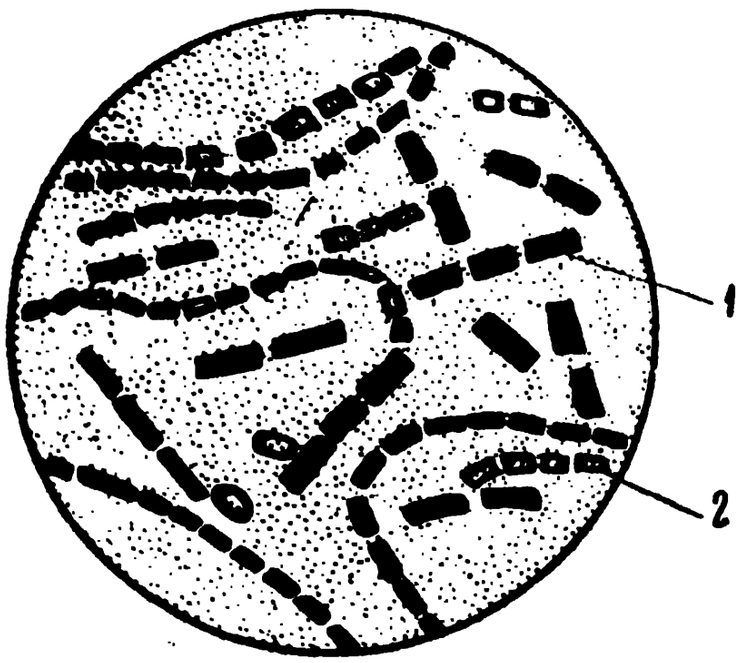

Fig. 12 Anthrax bacilli (*1*) and spores (*2*)

Therefore shape alone is not enough to identify a bacterium. To identify the microbe in question, numerous properties of the organism have to be established.

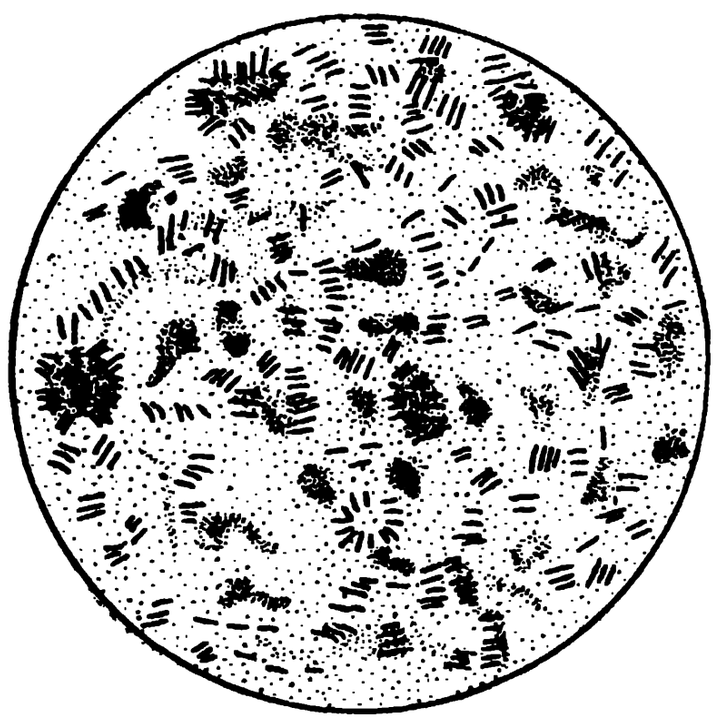

Fig. 13 Bacilli of leprosy

The cholera vibrio has one flagellum and is, therefore, active; spirochaetae, too, can move by twisting their bodies, like snakes. But the ability to move is not the rule for all bacteria. The dysentery rods, for instance, cannot move, neither can the anthrax bacilli move, for they have no flagella. Some types of bacteria, mostly rod-shaped, have organs of locomotion—the flagella. With few exceptions, *cocci,* too, are non-mobile.

Rod-shaped bacteria have certain other peculiarities. While observing with a microscope the *Bacillus subtilis*, the anthrax and tetanus* bacilli, we see inside some of the rods, or at one end, sparkling circular or oval excrescences. These are spores formed by some rod-shaped bacteria (Figs. 12 and 49).

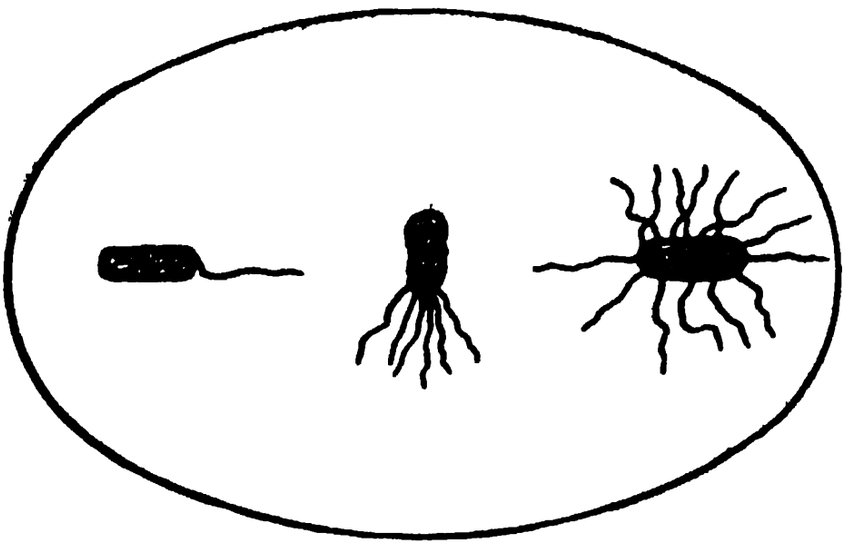

Fig. 14 Mobile rod-shaped bacteria with flagella

Bacterial spores are much more resistant than the vegetative forms** which produce them. Spores can do almost entirely without food and water and can remain alive for scores of years. Spores can withstand high temperatures, even boiling, and remain alive after heating to 212°F.,

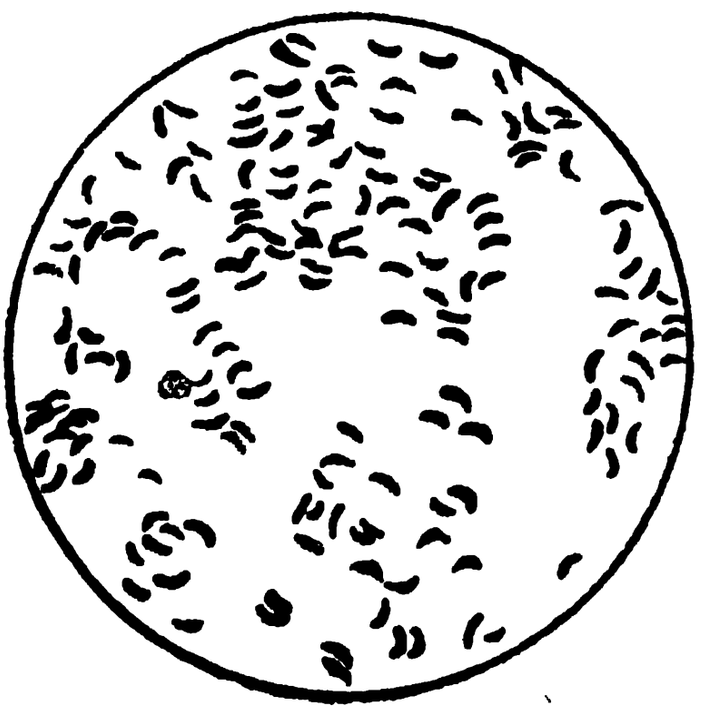

Fig. 15 Cholera vibrios

* See Chapter X.

** The vegetative form of a microbe is the state in which it feeds, grows and reproduces; bacterial spores are a form of rest adapted for preservation at a period when the bacterium does not feed, grow or reproduce

and even more. They withstand the action of light, are not destroyed by drying, for they have a very dense membrane and contain less water than the corresponding vegetative forms.

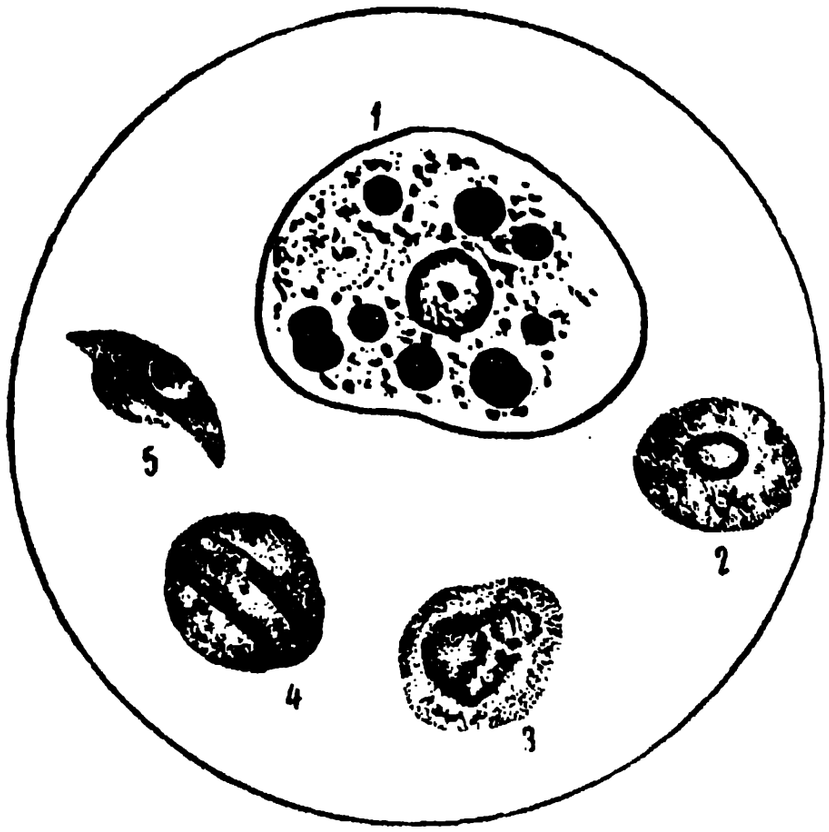

Fig. 16 Dysentery amoeba (*1*) and the malaria plasmodia (2—young; 3—adult parasite of tertian and 4—of quartan fever; 5—aestivo-autumnal

In a state of rest spores do not reproduce. No sooner, however, do they find themselves in favourable environment, e.g., the organism of man or animal (in case of the spores of pathogenic microbes) than they germinate, become transformed into the vegetative form, in which they feed, grow and reproduce normally. Consequently, spores formed by some rod-shaped bacteria are a defensive adaptation and contribute to the preservation of the species.

Now, let us examine under the microscope a drop of water out of a stagnant pool. Along with bacteria we

shall see in it unicellular protozoa—infusoria and amoebae. The majority of these are harmless, but there are amoebae which cause grave diseases. The inhabitants of southern countries sometimes suffer from an exhausting dysentery. The analysis of the patients' faeces reveals in it amoebae differing from the harmless ones inhabiting stagnant waters both in shape and in other properties. These are the causal organisms of the amoebic dysentery (Fig. 16, *1*).

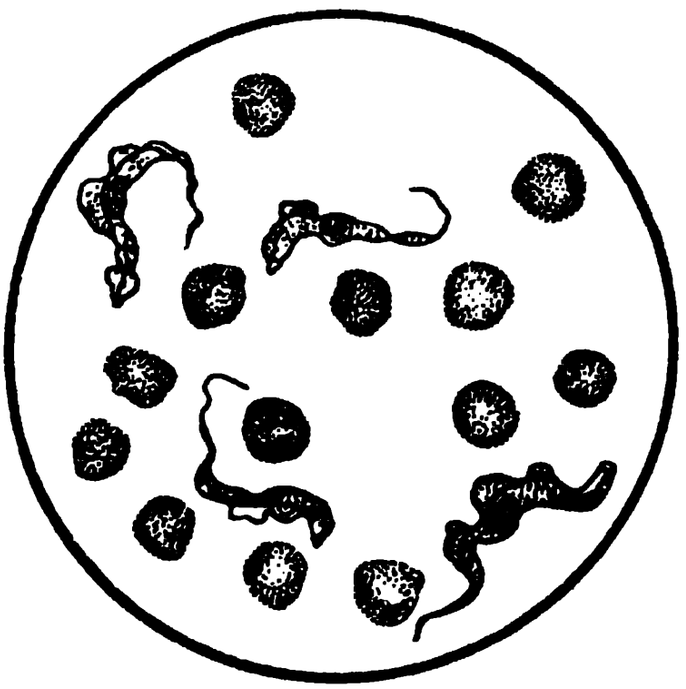

Fig. 17 Trypanosoma, the causal organism of sleeping-sickness

The blood of malaria patients (especially during an attack of the disease) contains protozoa, unicellular parasites called the malaria plasmodia, which are the pathogens of that disease. (Fig. 16, *2, 3, 4, 5.*)

In some parts of Africa people suffer from a lethal disease called sleeping-sickness. The blood of persons suffering from this disease contains certain infusoria—long, narrow, with a long tail and a transparent fringe along the body. This unicellular micro-organism is the *trypanosoma*, the causative germ of the sleeping-sickness (Fig. 17).

Although protozoa (amoebae, the malaria plasmodium, the trypanosoma, and others) are unicellular organisms, just like bacteria, they are much bigger. The size of protozoa varies from 2-5 to 60-70 microns, depending on the species.

If a piece of bread, fruit with punctured peel, or other foodstuffs are kept for some time in a damp, and

especially, dark place, a kind of whitish fluff appears on them, then the colour becomes brown, or the growth is bluish-green, or black. Seen under the microscope, this looks like a microscopic forest, consisting of tall columns crowned with spheric heads. This is the mould fungus *mucor*. When the head is ripe it bursts open and spores escaping from it either fall nearby or are carried by the wind in all directions (Fig. 18). Falling in places where there is dampness, food and little light, the spores germinate and give rise to a new *mucor*, to a new colony of intertwined thin fibres (hyphae), called *mycelium*. These propagative spores do duty for seeds, i.e., are organs of reproduction and have nothing in common with the spores of bacteria, which perform the defensive function, except the name. But there are also resting fungus spores which perform the function of preserving the species during periods unfavourable for vegetation—for germinating into mycelium, feeding and reproduction.

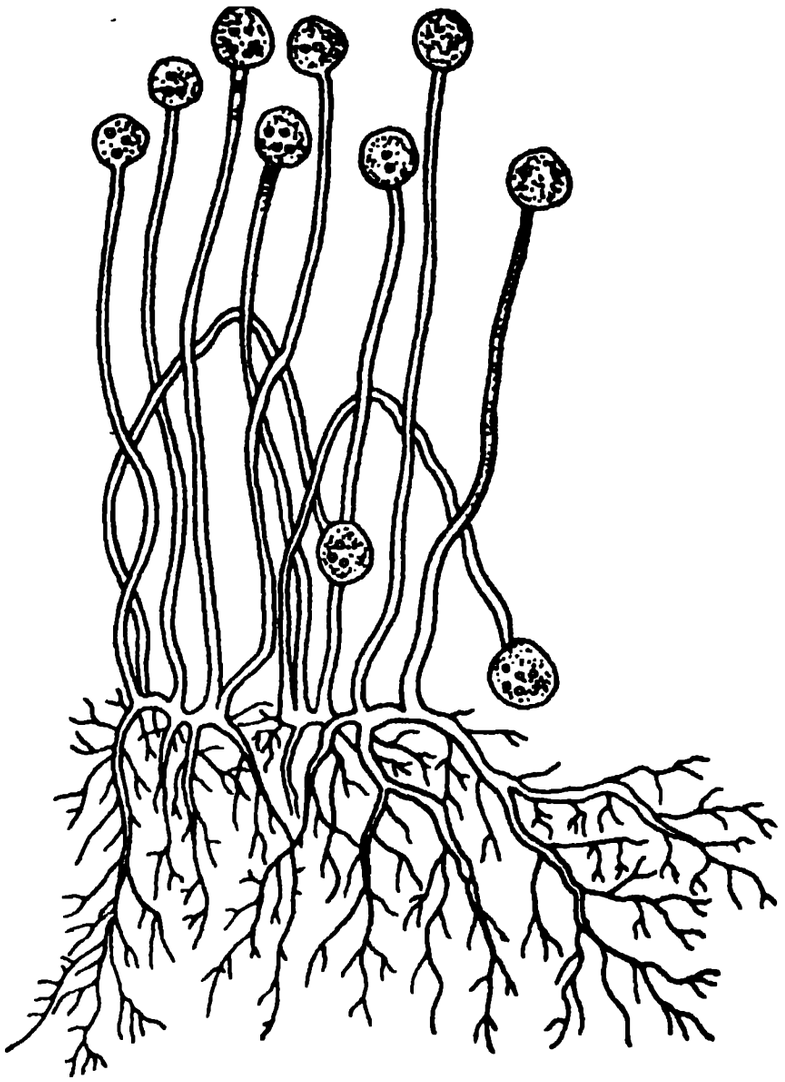

Fig. 18 Mould fungus *mucor*

If we examine under a magnifying glass the bluish-green mould on a rotten apple we shall also see a my-

celium but the hyphae will have at the top something resembling a hand with fingers spread out. A closer study will show that the "fingers" consist of separate beads—conidia—strung together, like a rosary. When

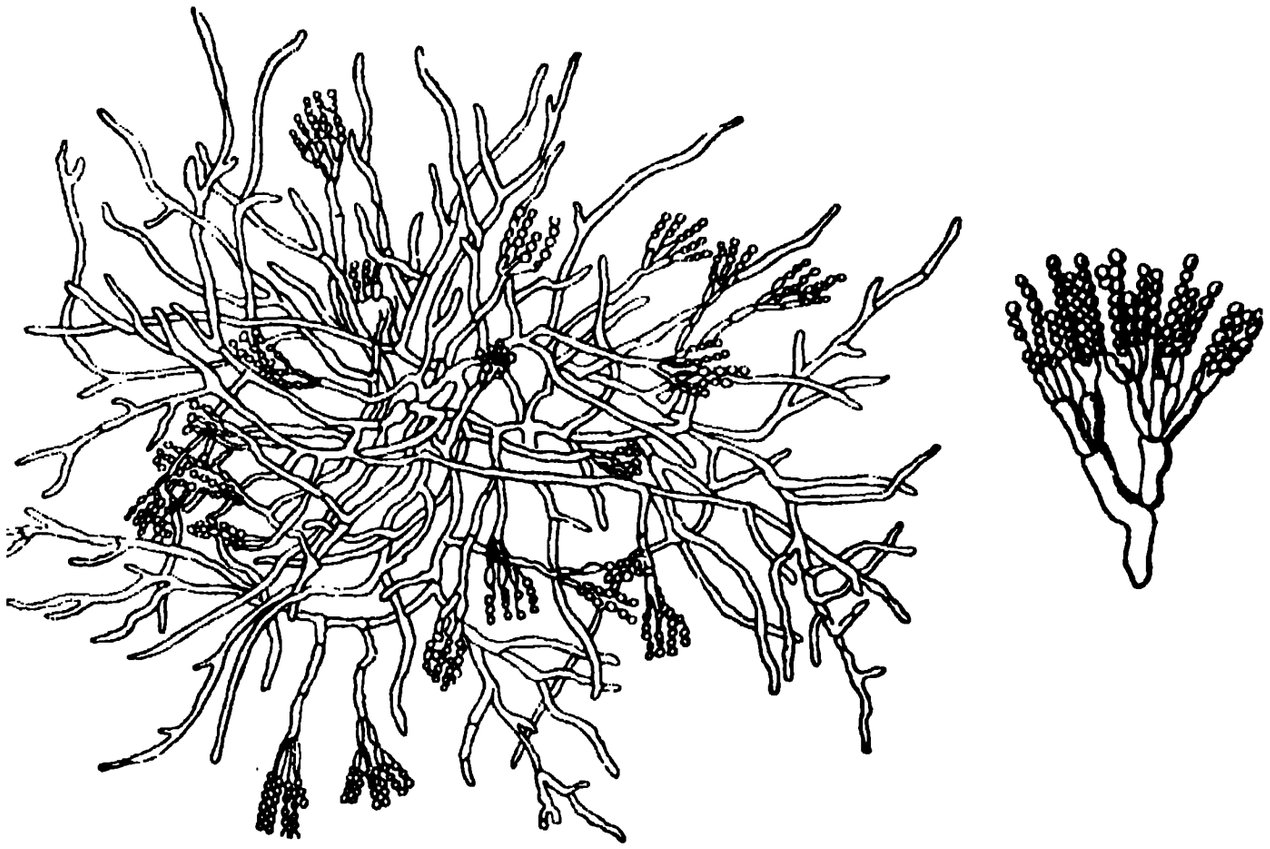

Fig. 19 Mould fungus *Penicillium glaucum*

the conidia ripen they separate from the rest and either drop off or are carried away by the wind, giving rise to new mycelia wherever the conditions are favourable. This is the *Penicillium glaucum* known in everyday life as green mould. Its conidia perform the function of reproduction just as do the spores of *mucor* (Fig. 19).

Now let us take a piece of baker's yeast, add some water to it and examine a drop of this liquid under the microscope. We shall see circular and oval cells, some of them with round excrescences of various sizes. These are yeast cells with "buds"—the future generation of the yeast fungus. Grown buds will either leave the mother cells or remain connected to them, forming colonies like those of corals (Fig. 20).

Many species of the yeast fungus are useful (see Chapter V): there are baker's yeast, brewer's yeast, wine yeast, and some others, differing from one another not so much in shape as in properties.

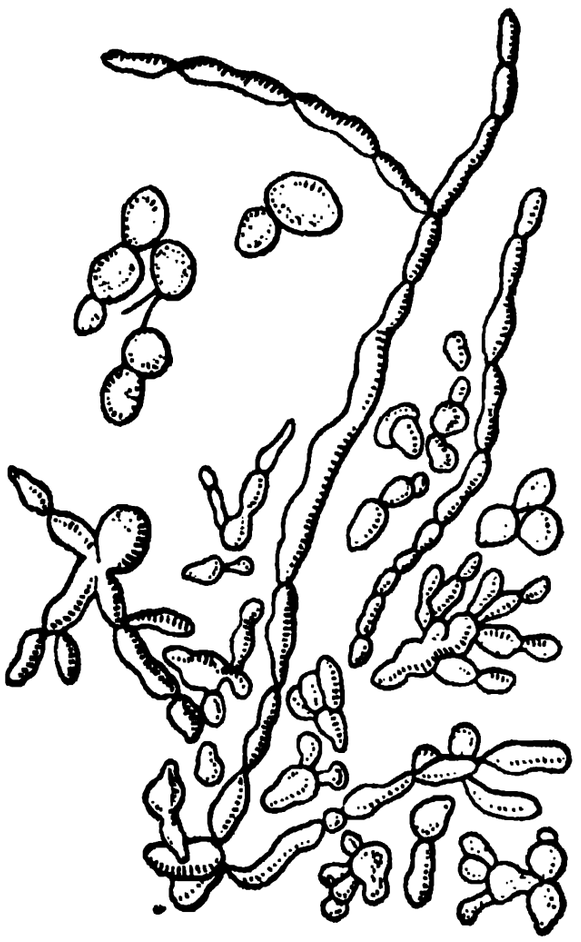

Fig. 20 Yeast fungi propagating by gemination

There are also microscopic fungi causing various diseases in man, animals and plants, such as the fungus attacking the hair on the head of a human being and causing it to fall off. A person may become temporarily bald, but if the disease is neglected new hair may never grow. This disease is called mange and it usually attacks young people. There are other fungi causing diseases like mange, as for instance, the ring-worm.

The ray fungus—actinomyces—also occurs abundantly in Nature. Some species of it are pathogenic, causing actinomycosis in animals, mostly in cattle, but sometimes in man as well. It usually affects the lower jaw, sometimes the tongue, lungs and pleura. Actinomycosis begins with a tumour which gradually softens; then appears a deep fistula from which exudes yellowish pus containing grain-like *drüsen.*

Among mould, ray and other fungi there are useful species, from which antibiotics are extracted—preparations enabling us to fight many infectious diseases. (This problem is treated at greater length in Chapters III and XI.)

Typhus is caused by a species of bacterium-like microbes called rickettsia, after Ricketts who identified them. Besides those causing typhus, dozens of other rickettsiae are known; these are the parasites of various insects and, except a few species, are harmless for man. From Fig. 21 we can see that rickettsiae have different shapes—some are globular (*cocci*), others are like dumbbells, rods, or long wavy threads.

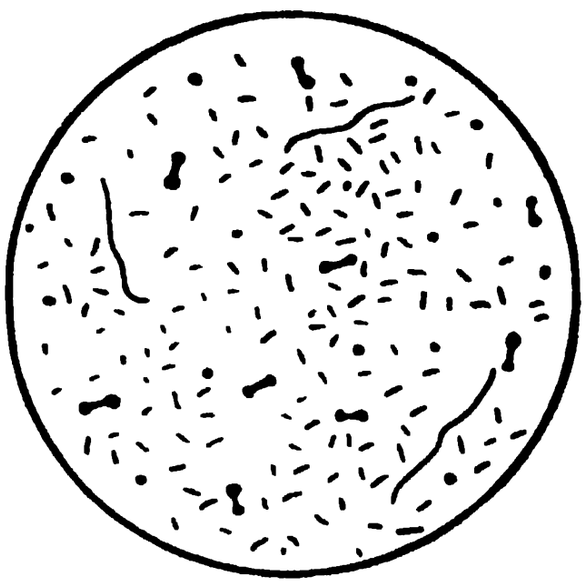

Fig. 21 Rickettsiae

Speaking of rabies in Chapter I we mentioned that Pasteur, who found the means of combating the disease, could not discover its causal organism. Today this would not seem astonishing for we know that the organism is a filtrable virus, and filter-passers are much smaller than bacteria. The virus of rabies cannot be seen through the ordinary microscope, even a very powerful one.

If the blood of an animal affected with barbs or cattle plague is passed through the bacterial filter it will remain as infectious as before, because the causative organism of the disease is a filter-passing virus.

There is an immense variety of filtrable viruses in Nature, which cause numerous diseases in man, animals, birds, insects and, particularly, in plants. Recently methods were found of measuring viruses. To imagine the size of filter-passing viruses, we must bear in mind that a millimicron (m μ) is as many times smaller than a millimetre (mm) as a millimetre is smaller than a kilometre.

Some viruses are relatively large as, for example, the Paschen bodies, the smallpox organisms. In shape they resemble bricks and stones with polished edges; their

size ranges from about 244 to 300 mμ, and when dyed the bodies can be seen through the ordinary microscope (Fig. 22). The size of some filter-passing viruses is no more than a few millimicrons.

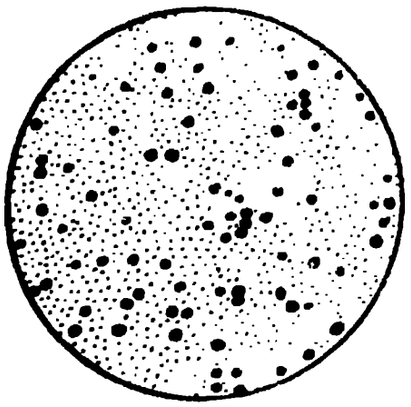

Fig. 22 Paschen bodies seen under an ordinary microscope

The invention of the electron microscope has made possible the study of many filter-passers.

Like all other living beings, microbes require food to grow and multiply. In spite of their microscopic size, microbes have a very complex chemical structure. The protoplasm of microbes consists of proteins, carbohydrates, fats and fat-like substances, and mineral salts. Many of these substances are in a state of colloid solution, the water making up 80 to 85 per cent of a microbe's body.

In addition, microbic cells contain ferments or enzymes, vitamins, and vitamin-like substances. If we compare the elements of which microbes and more complex organisms, including animals and man, are composed, we shall see that the difference lies only in quantities, in the proportion in which these elements are combined, and in mammalia possessing different organs for the chief biochemical functions,

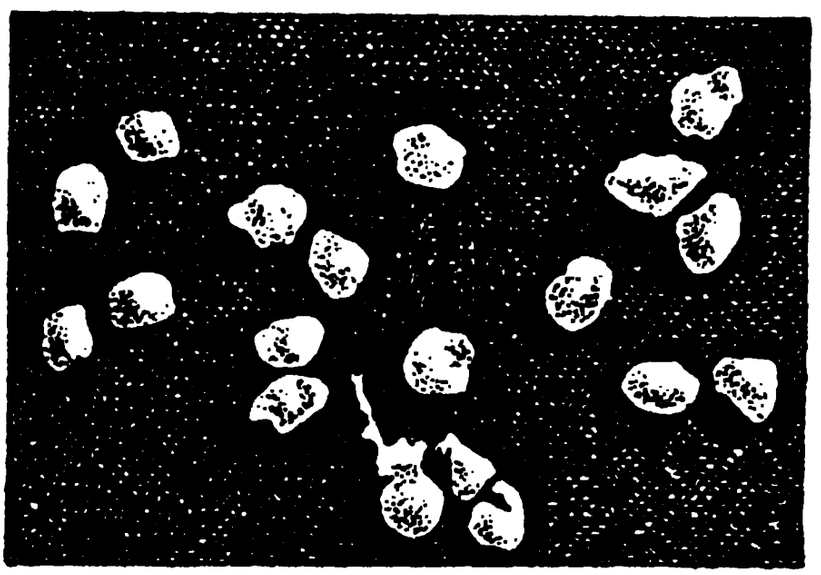

Fig. 23 Paschen bodies seen under an electron microscope

while in microbes all functions are performed by the protoplasm of their one-cell organisms. Indeed, as N. Yerusalimsky put it: "All living organisms are cut from the same stuff but made to different fashions."*

This shows that the nutrition requirements of micro-organisms are as complex as those of higher animals.

How do micro-organisms absorb their food? The water solutions of salts and sugars they need are easily assimilated by the protoplasm. But many microbes need also proteins, fats and some other substances which are not soluble in water. These must be in a state in which the protoplasm can assimilate them. This process is facilitated by ferments or enzymes which are part of microbes' constitution.

Ferments or enzymes have some specific properties which are very important for living organisms. Minute quantities of ferments can produce radical changes in great masses of organic complexes. Ferments themselves seem to take no part in the reaction, they merely activate the process. Ferments function at the temperature of the body of animals, or some other living organisms, and even at the temperature of plants, which is lower.

Ferments are specific, each ferment acting on a definite substance (substrate), or part of it, therefore, to be able to assimilate food, every organism has a set of specific ferments.

Proteases, for instance, decompose proteins, and different other ferments are needed to decompose sugars. Beet sugar is decomposed by saccharose, lactic sugar by lactase, etc. Lipoids—fats and fat-like substances—are decomposed by lipase and other ferments.

The chemical composition of microbes is rather complex, and a microbic cell contains a great variety of ferments. The conditions in which a microbe lives and the food it consumes determine its set of ferments.

* N. Yerusalimsky, *Nitrogen and Vitamin Food of Microbes*, U.S.S.R. Academy of Science Publishing House, 1949.

Microbes need ferments not only for making proteins and other substances soluble and, consequently, digestible. Suppose that all food substances have been dissolved in water and have been absorbed by a microbe body. That would not be enough, for the substances have to be assimilated by the microbe, i.e., utilized for its growth and propagation, and that is exactly what ferments help to do.

Hence microbes, like bigger organisms, including man, could not exist without ferments.

But ferments alone are not enough for a microbe to live and develop normally. There are other substances without which microbes, just like multi-cellular organisms—animals and man—could not grow and reproduce, namely, vitamins. Everybody knows that lack of one or another vitamin in an organism causes various diseases. Thus the lack of vitamin C causes scurvy, the lack of vitamin D in children causes rachitis; the lack of vitamin B_1 in man causes polyneuritis. This latter disease, called beri-beri in the East, develops from a diet of polished rice, because rice husk contains vitamin B_1. Micro-organisms, too, cannot grow and develop normally if they lack vitamins.

Micro-organisms receive part of the vitamins they need from food and the rest they produce themselves. Not all vitamins, however, can be produced by micro-organisms, and not all microbes possess this ability in an equal degree. Soviet scientists have observed cases of complete inability to synthesize vitamins in some pathogenic bacteria and in protozoa.

Most of the microbes can be subdivided into groups on the basis of the food they need. Some, as for instance, pyogenic and pathogenic microbes, require ready-made organic compounds, others—and these are more numerous—do not need organic compounds, as they are capable of assimilating mineral (non-organic) compounds, and even such elements as atmospheric nitrogen.

It is not always and everywhere that a certain variety of microbes finds the food it requires. In such cases they have but a meagre diet and sometimes endure hunger. In the world of microbes, as among other living beings, the best adapted individuals survive unfavourable conditions. Many microbes can adapt themselves to most varied environmental conditions.

Some micro-organisms subsist on substances absolutely unfit and even poisonous for other living organisms. Sulphur bacteria, for instance, feed on hydrogen sulphide, upon the assimilation of which sulphur is deposited in their bodies. The urea bacterium lives in ammonia and feeds on nitrogen contained in it. We know of microbes subsisting on petroleum, paraffin, naphthalene, etc.

Although the invisible organisms are ubiquitous, since they adapt themselves to all sorts of conditions, all of them are divided into two groups—saprophytes* and parasites, depending on their mode of life.

Today by saprophytes we mean the greater part of microbes existing outside living organisms.

Parasites, micro-organisms existing within living organisms at the expense of the latter and injurious to them, are opposed to saprophytes.

All pathogenic microbes causing infectious diseases are parasites.

Many parasites, however, can, for a time, behave like saprophytes, i.e., live not within a host—a human, animal, insect, or plant organism—but on non-living substances, on which they feed, grow and proliferate. Parasites can live for a considerable time in reservoirs with a scant supply of nutritive matter. The ability of some pathogens to exist outside the host renders them

* From *sapros*—putrid and *phyton*—a plant, i.e., micro-organisms developing in putrid vegetable remains. Practically all non-pathogenic micro-organisms living on vegetable and animal remains are called saprophytes.

more dangerous than the microbes which do not possess this property.

The abundance of micro-organisms in Nature is due to the great variety of substances they thrive on and to their adaptability.

This will be easier understood after a study of the reproduction of micro-organisms and, particularly, of bacteria, when we know their activity and the influence that different temperature, humidity and light exert on them.

* * *

Every living organism has to have energy in order to feed, move, multiply and perform other vital functions. Micro-organisms are no exception in this respect.

But where do they obtain energy? The source of energy for micro-organisms is the decomposition and oxidation of the substances they use for feeding, growing and reproduction. Often one and the same compound may be used both as the building material for the cell and the source of its energy. The substances oftenest used for supplying energy to microbes are those containing no nitrogen.

Micro-organisms are able to assimilate not only carbohydrates (sugars) but also other organic substances, which they oxidize in order to obtain energy for vital processes. The microbes utilizing for this purpose atmospheric oxygen, for instance, the vinegar bacillus (see Chapter V), some moulds, etc., are called aerobes.

But there are many microbes which do not need atmospheric oxygen for their vital processes. They obtain energy by decomposing various substances containing hidden stores of energy. Such microbes are called anaerobes. To this category belong the tetanus bacillus, the botulinum bacillus (see Chapter X), and many micro-organisms occurring in soil.

Besides micro-organisms which are strictly aerobic, i.e., which cannot live without atmospheric oxygen, and the obligate anaerobes, i.e., such as can live only where there is absolutely no atmospheric oxygen, there are many microbes which are both aerobic and anaerobic, i.e., can live where there is the usual quantity of atmospheric oxygen, where there is little oxygen and even none at all—under the anaerobic conditions. Thus the typhus bacterium which is an aerobe can live without air. Yeast fungi can perform vital processes, i.e., can decompose glucose (grape sugar) under both aerobic and anaerobic conditions. But in the presence of atmospheric oxygen the decomposition proceeds to the end—until water and carbon dioxide are obtained—while within wine must, where air does not penetrate, the process goes only as far as the production of carbon dioxide and alcohol.

* * *

We know that a sure method to prevent infection with intestinal diseases is to boil drinking water, because boiling kills most of the bacteria, including the pathogenic organisms in water. The only exception are spore-forming bacteria.

Typhoid-fever, dysentery and other bacteria causing intestinal diseases form no spores and are destroyed by boiling. Most of the non-sporulating bacteria die at temperatures between 140° and 176°F. As has been noted elsewhere, heating at these temperatures constitutes pasteurization of beer, wine, milk, etc.

But there are many microbes which cannot be destroyed in this way, for they can develop at 122°F. and even 158°F.; they are known as thermophilic, i.e., warmth-loving, microbes. And lastly, there are microbes which do not require any warmth and thrive at temperatures below 64.4°F., and even below 50°F. Such microbes can multiply at temperatures below 32°F.

and they are found in northern seas, ponds, lakes and rivers, and also in refrigerators. Thus, mould fungi can develop slowly on meat and butter at 14°F. and less. Such microbes are called psychrophilic, i.e., cold-loving.

The majority of micro-organisms, however, thrive at temperatures between 68°-77°F. (sometimes less) and 104°-113°F. All pathogens belong to this group, since the best temperature for these is that of the human or animal body, between 96.8° and 104°F.

Experiments determining the influence of high and low temperatures on micro-organisms have shown that even thermophilic microbes cannot endure temperatures above 176°F. At higher temperatures their vitality gradually ebbs, until death occurs. Some microbes die at 131°F. to 140°F. A temperature of 212°F. destroys all but sporulating micro-organisms because at this temperature proteins in their protoplasm coagulate, which causes death.

Spore-forming bacteria are destroyed in the autoclave* by treatment at a great pressure and at a temperature of 248°F. for 30 minutes and more.

In a gradually falling temperature the vitality of microbes ebbs with the dropping of the temperature below the optimal, until it becomes suspended. But low temperature does not invariably destroy microbes as is the case with heat at 212°F. The temperature may be reduced very considerably, the microbes may freeze, but many may still survive.

Experimental freezing of microbes at temperatures of —310°F. and even at —426°F. has shown that after gradual heating the microbes revived. Hence, freezing

* The autoclave is a boiler with a hermetically closing lid Water heated in the boiler evaporates and the steam produces a pressure above the atmospheric.

only suspends the vitality of many microbes but does not kill them.

Microbes live and develop in a liquid medium. Deprived of this medium, most of the microbes, including putrefactive ones, become inactive and cease to develop. That is why, to preserve meat, fish and fruit, they are dried.

Many microbes, however, are highly resistant to drying. As soon as dried micro-organisms encounter favourable conditions, they absorb the necessary amount of moisture and develop anew.

This property of microbes has enabled scientists to preserve dried microbes in laboratories and to transport them in this state from one institution to another.

The Soviet scientist V. Tauson has found large quantities of various bacteria, mostly cocci, in absolutely dry and arid sands and rocks of the Pamir highlands. His observations have shown that these bacteria remain in a dried state from six to eight months a year.

When dried-up but still viable microbes get in places where there is moisture and food, they absorb water till their protoplasm contains the necessary percentage of it and again start feeding, growing and reproducing.

As a rule microbes are not destroyed by dispersed sunlight. But sunrays falling directly on microbes kill them in about two odd hours, depending on the resistance to sunrays possessed by different micro-organisms. Microbes containing a normal quantity of water are destroyed by direct action of light much quicker than dry microbes and the difference in time is still more marked in the case of spores. That is why dried-up but viable microbes are found on the surface of granite rocks which are heated by directly falling sunrays to a temperature of 140°F. to 158°F.

* * *

How do micro-organisms proliferate?

Bacteria and other micro-organisms usually reproduce by division. A bacterium grows to a certain limit and is then divided, producing two new bacteria. Most of the yeast bacteria reproduce by budding. Some yeasts produce several spores within their cells. When old cells disintegrate the spores come out. Under favourable conditions they germinate and become young yeast cells. It would be interesting to follow the rate at which microbes, particularly bacteria, multiply, which is different for different microbes. On the average a bacterium, under favourable conditions, divides in 30 minutes. Consequently, in 30 minutes each bacterium produces 2, in an hour 4, in an hour and a half 8, in two hours 16, in 3 hours 64, in 4 hours 256, in 5 hours 1,024, in 10 hours 262,144 and in 20 hours 191,667,200,000 bacteria, whose aggregate weight would be 80 mg. In 40 hours the bacterium colony must weigh 18,841.6 tons.

The rate at which the cholera vibrio proliferates is so high that in 30 hours the descendants of one vibrio could cover the face of the globe in one unbroken layer.

Other microbes, such as yeast fungi and moulds, multiply at a slower rate but much quicker than organisms visible with the naked eye, and especially animals.

In spite of this extraordinary ability to proliferate which might apparently lead to an incredible number of micro-organisms filling the whole world and extinguishing all other life on earth, no such catastrophe has ever happened or is likely to happen in the future.

The proliferation of bacteria is checked by lack of nutritive matter and by other adverse circumstances. Although not always fatal to microbes, high or low temperatures and drying often hold up their reproduction for a time. Besides, larger organisms devour enor-

mous quantities of microbes. And finally, vast numbers of microbes perish as a result of the existing antagonism between different micro-organisms.

All these unfavourable circumstances prevent microbes from proliferating on a disastrous scale. But if a microbe finds nutrition and other favourable conditions it can produce many millions of individuals in two or three days.

A large number of micro-organisms will be found in the human mouth, on the skin surface and still more in the intestinal tract of both man and animals. Many microbes have been discovered in the bowels of insects and on their integuments. Micro-organisms abound on the roots of plants, inside the roots of leguminous plants and occur even on ice.

Soil, however, contains the bulk of all micro-organisms and from there they spread to water, the air and to all objects on the earth's surface. Ponds, lakes, rivers and wells always contain micro-organisms in a greater or lesser quantity, most of which are washed into the reservoirs from the shores or banks.

Numerous investigations have shown that rivers contain the greatest number of microbes when passing through and leaving city precincts. This is because rivers in the vicinity of human habitation receive sewage and industrial waste, and their banks are usually thick with refuse. Here people wash their linen, bathe and water their cattle.

The farther away a river is from a populated locality, the purer is its water, and the fewer the microbes in it, for pure water usually contains little food for them.

Water contaminated with microbes can cause typhoid fever, paratyphoid, dysentery, cholera and other infectious diseases, because pathogenic bacteria get into the water from the excretions of humans and animals.

We know that there is always dust in the air. In some places there is more dust, in others less. Dust comes from the soil and always contains microbes in a greater or smaller quantity.

Dust forms also with the drying-up of human and animal excretion. When a consumptive person coughs up sputum containing tubercular bacteria, it dries up and, together with microscopic particles of soil, is turned into dust. Live tubercular bacteria sail on specks of dust in the air which becomes dangerous for healthy people; a person can contract tuberculosis by breathing in air containing contaminated dust.

Although there are not so many microbes in the air as in soil, water or surrounding objects, the number is considerable, especially in the air of large cities and inside stuffy premises.

Numerous bacteriological tests of air, dating back to Pasteur's time, have shown that the quantity of micro-organisms in the air depends on the location of a given place. The farther it is from towns and cities, e.g., far up in the mountains or in the Arctic, the fewer microbes there are in the air.

But no spot on land or in water is entirely free of microbes. They are everywhere—high up in the mountains, in arid deserts, in the Arctic depths and all other seas, in mines several miles deep.

As V. Omelyansky wrote in *The Foundations of Microbiology*: "They are truly ubiquitous: myriads of microbes populate the elements and are everywhere round about us. Unseen, they accompany man throughout his lifetime, sometimes as friends and sometimes as enemies. They occur in great numbers in the food we eat, in the water we drink, and in the air we breathe. All objects around us, our clothing, our bodies—everything teems with microbes."

From time immemorial micro-organisms have performed varied functions in Nature. Curative muds,

petroleum, combustible gases, red lakes and pink salt, carbonate of lime, all owe their formation to the activity of micro-organisms.

* * *

Let us consider the properties of some species of micro-organisms. Observations of bacterial cultures have shown that bacteria form colonies which, depending on the species, are differently coloured or are colourless. The colouring comes from the pigment in their bodies.

Off the coast of the Kola Peninsula in the Barents Sea, in Kolsky Straits near Murmansk and in several other places, the sea is red. In the estuaries of the Black Sea near Odessa and in some salt lakes the water, too, is reddish. This colouring is due to the action of certain species of sulphur bacteria inhabiting these waters, which are known as "purple bacteria," because they produce a red pigment. The rod-shaped bacterium *pseudomonas pyocyanea* lends a blue colouring to putrescent wounds in which it develops. There is the "blue milk rod," a bacterium which develops in milk and lends a bluish tint to it.

A species of staphylococci produces a golden-coloured pigment. The layer of mould developing on foodstuffs is almost invariably of a bluish-green, black, dark brown, or other colour, depending on the pigment produced by a given species of *mucor*.

In ancient Greece and Egypt priests forbade the use of white beans for food, because soon after cooking they "turned into blood," i.e., became red.

In 1329, in some German towns, the hosts in Catholic churches "became covered with blood spots." A superstitious fear gripped the Catholics; rumours spread that the bleeding of consecrated wafer was God's visitation. Priests accused "heretics" of witchcraft and demanded blood for blood. All this ended in the burning at the stake of about 10,000 innocent victims in Frankfort, Würzburg, Nuremberg and other towns.

Once again "blood spots" appeared on hosts in a church at the small German town of Wilsnack in 1383. They were washed away, but soon re-appeared. Fresh hosts were substituted for the "bleeding" ones but these, too, were soon spotted with "blood." The Wilsnack "miracle" again brought about the persecution and burning of the "heretics" who were charged with pricking the hosts which then began to bleed.

More than 400 years had passed before any light was shed on this strange phenomenon. In the house of Pitarello, a peasant in Legnaro near Padua, northern Italy, "blood spots" appeared on bread in 1819. The terrified peasant sent for the priest but his prayers were of no avail. Crowds of people from Legnaro and nearby villages came to Pitarello to see the "miracle"; rumours spread far and wide.

This, too, might have ended in disaster, as in Germany in the 14th century, but, fortunately, Sette, the local doctor, was a clever and well-educated man. He thought these spots were like mildew growth. To make sure, he took some of the "bloody" matter and infected with it fresh foodstuffs, on which similar growth appeared. He repeated the experiment in the house of the priest who, evidently, was loved by his parish, and the results were the same. In this way the doctor pacified the population and prevented violence.

Later it was discovered that a microbe called *Bacterium prodigiosum* develops on foodstuffs with a high starch content. When big colonies of the bacterium develop they cover the product with a red investment resembling spots of blood.*

* * *

* The above examples are taken from *The Foundations of Microbiology* by V. Omelyansky, Uchpedgiz Publishing House, 1936.

"While sailing a little south of the Plata on one very dark night," Darwin says in his *Voyage on the "Beagle,"* "the sea presented a wonderful and most beautiful spectacle. There was a fresh breeze, and every part of the surface, which during the day is seen as foam, now glowed with a pale light. The vessel drove before her bows two billows of liquid phosphorus, and in her wake

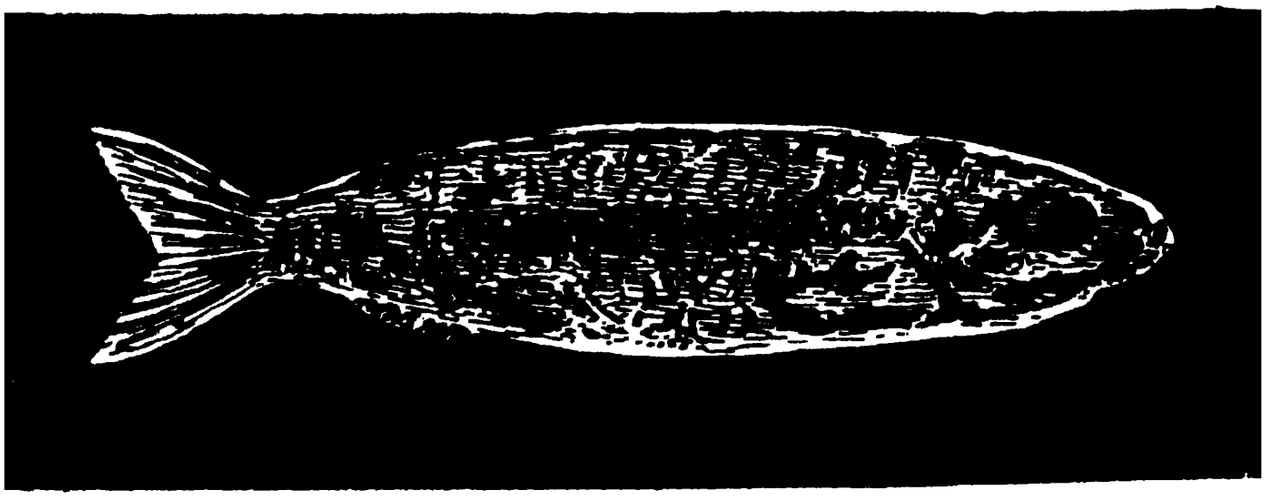

Fig. 24 Herring lighted by the photobacteria developing on its body

she was followed by a milky train. As far as the eye reached the crest of every wave was bright, and the sky above the horizon, from the reflected glare of the livid flames, was not so utterly obscure as over the vault of the heavens.... On two occasions I have observed the sea luminous at considerable depths beneath the surface."

Many famous scientists and travellers including F. Nansen, C. Linnaeus and Amerigo Vespucci have described the phosphorescence of the sea.

B. Isachenko, A. Yegorova and other Soviet scientists have established that the phosphorescence of the sea is caused chiefly by the presence of photobacteria* which

* V. Omelyansky points out that the sea may fluoresce owing to the presence of the infusorian *Nocticula miliaris*. This phenomenon may also be caused by the *Lampyris nocticula*, a beetle of the genus *Pyrophorus* occurring in South America, and by other organisms.

usually develop on the bodies of medusae, mollusca, some fishes, small sea animals, etc., and some of which are washed off and continue to scintillate in the water. A case has been described of a drowned body becoming luminescent after staying some time in the sea.

Fig. 24 shows a herring photographed by its own light emitted by slime on its body, that is to say, by the myriads of bacteria developing in the slime.

In one of his works B. Isachenko described a fatal disease of insects (gnats) caused by fluorescent bacteria.*

Fluorescent bacteria have been discovered on meat and other foodstuffs.

* B. Isachenko, *Selected Works*, Vol. I, U.S.S.R. Academy of Science Publishing House, 1951. pp. 59-60.

Chapter III

SOIL, PLANTS AND MICRO-ORGANISMS

The productive capacity of soil is its most important property. Academician V. Williams said that "Productivity is inherent in the notion 'soil.'" Only that soil is good which supplies plants with nutriment in a sufficient quantity. But even good soil will not yield a good harvest without tilth, the purpose of which is to create the best conditions for the development of plants.

In the old times a land tiller could only learn from the experience of past generations. But in the course of time scientists began studying the life of soil in order to discover reasons for good crops as well as for low productivity.

New sciences appeared, such as agronomy and soil science, to which Russian and Soviet scientists made substantial contributions.

Investigations have shown that plants need many different substances containing nitrogen, carbon, phosphorus, potassium and a number of other elements for their growth and nutrition. There is an abundance of these substances in the soil but they are usually in such a state that plants cannot utilize them directly.

The question arose: How do plants manage to extract from the soil the substances they need?

The first to provide an answer to this question were the Russian scientists V. Dokuchayev (1846-1903) and P. Kostychev (1845-95), who found that substances contained in the soil are transformed by micro-organisms

into such compounds as can easily be absorbed by the roots of plants.

Under the action of micro-organisms dead plants and animals decompose, producing simple inorganic compounds. But to be able to perform such titanic work, there must be enormous numbers of micro-organisms in the soil.

And indeed, as was established in the past century, soil abounds in micro-organisms. Further investigations showed that one gramme of soil often contains as many as several thousand million of various microbes. But they are not distributed in the soil in equal quantities: the greatest number occurs in the upper layers, but as we go deeper, the number of microbes diminishes. The bulk are found around and on the roots, in what is known as the rhizosphere, where they are engaged in converting the elements of the soil to plant-available forms.

The weight of a microbe, especially of a bacterium (which prevail in soil), is infinitely small, but the total weight of the micro-organisms in a hectare* of ploughed land is from 5 to 15 tons. The aggregate weight of all microbes, counting the dead but not yet decomposed organisms, must be much greater.

It is not the weight, however, that matters but the vast active surface area of the microbe population, which is equal to nearly 500 hectares in one hectare of soil.

Soil organisms produce various and very active ferments which cause profound changes in complex organic and inorganic substances of the soil. The microbes themselves use for food, growth and proliferation the simple compounds which appear as a result of the decomposition of organic substances and serve as food for plants.

The myriads of dead microbes, too, decompose and change into simple inorganic compounds which are then

* A hectare is equal to 2.47 acres.

used by plants and new generations of microbes.* Bacteria and fungi in the soil decompose vegetable and animal remains and produce humus.

Academician V. Williams and other scientists have established that fine-structured soils are the best for plants. In order that such soils may develop there must be some kind of adhesive and, indeed, humus, freshly produced by micro-organisms, envelops soil particles and cements them together in small aggregates.

Thus we see that soil is not only the medium in which microbes live but is the result of their activity.

Lately Soviet scientists (particularly N. Krasilnikov) have proved that plants can assimilate complex compounds, such as vitamins, antibiotics, and so forth. Therefore, these substances may play an important part in plant life. There is a chance, too, that through their root system plants can assimilate certain quantities of other organic compounds.

Besides an abundance of bacteria soil contains various fungi, some of which form on the roots of plants an excretion called mycorrhiza (from the Greek *mykes*—fungus and *rhizos*—root). Mycorrhiza is a fungous mycelium developing in plant roots.

In some cases the growing part of the roots is encased in a more or less solid fungous sheath from which stretches a network of hyphae. The root hairs die off and water and nutritive substances are absorbed through the mycelium, the hyphae of which stretch from the roots to the soil. Sometimes the mycelium of the mycorrhiza develops mainly in the tissue of the bark of the roots, and little of it is visible on the surface. The mycelium invasion may be inter- or intra-cellular.

Mycorrhiza develops on many trees, as for instance

* E. Mishustin, *The Dokuchayev-Kostychev-Williams Complex and Soil Microbiology, Courier of the U.S.S.R. Academy of Science*, No. 12, 1951.

coniferous, oak, hornbeam, beech, and other higher plants, including herbs. Generally speaking most trees and herbs in all the zones of the globe live in a symbiosis with fungi. Almost all agricultural plants have mycorrhiza.

Science has not yet been able to establish why in the absence of mycorrhiza many higher plants develop but poorly or do not grow at all; Soviet scientists presume that the beneficial role of mycorrhiza is due to the following: mycorrhiza decomposes complex organic and inorganic matter until simple inorganic compounds are obtained, these are assimilated by plants through the ramified network of the mycorrhiza mycelium which heightens the ability of the roots to assimilate nutritive matter and water.

The symbiosis enables the mycorrhiza fungus to utilize some substances contained in the plant roots.

The formation of mycorrhiza is of special importance in forest planting. In order to have mycorrhiza in young trees scientists recommend the artificial "mycorrhization" of field-protective forest belts, by introducing into the soil small doses of forest soil rich in beneficent fungi.

* * *

Let us consider at some length how microbes help plants to assimilate nutritive elements such as nitrogen, phosphorus, potassium, carbon, etc.

Nitrogen is easily assimilated by plants in the form of the ammonium salts which are produced as a result of decomposition of animal and vegetable remains, and also in the form of nitrates. Ammonium compounds are converted in the soil into nitric acid by the nitro-bacteria (Fig. 25) discovered by S. Vinogradsky (see Chapter I).

There are two species of these bacteria: nitrite bacteria oxidizing ammonia to nitrites and nitrate bacteria, oxidizing nitrites to nitrates. Nitro-bacteria are active

only in the presence of oxygen in large quantities, i.e., at sufficient aeration which is achieved by a good tillage of the soil.

Nitrogen contained in the urine of man and animals is also assimilated by plants with the assistance of micro-organisms.

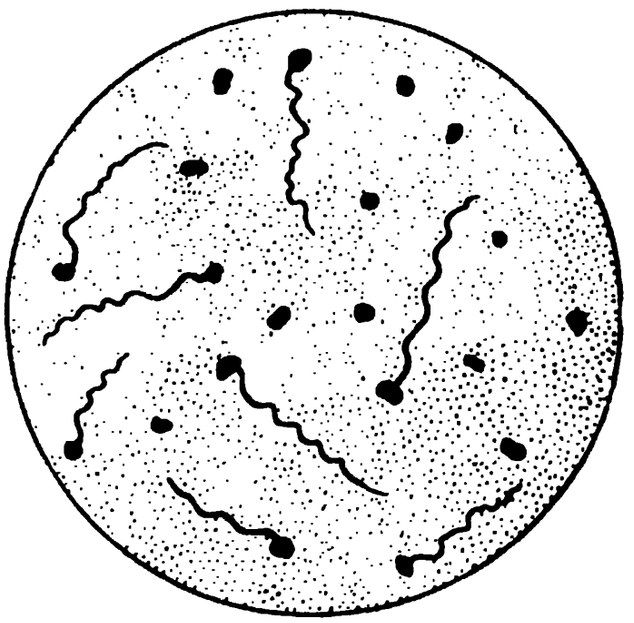

Fig. 25 Nitro-bacteria

A man or an animal excretes daily from 30 to 50, and more, grammes of urea which is rich in nitrogen. But plants cannot utilize urea nitrogen directly. In this they are helped by bacteria; the urea bacteria split urea, producing the ammonium salts, then nitro-bacteria produce nitrates which can be assimilated by plant roots as easily as ammonium (Fig. 26).

In addition to nitrogen contained in the soil, plants receive it from fertilizers, such as manure, peat and mineral nitrogenous fertilizers.

Investigations made by D. Pryanishnikov have shown that cereal crops absorb enormous quantities of nitrogen. It is clear, therefore, that the richer a crop the more nitrogen it takes from the soil.

Part of the soil nitrogen is wasted without any benefit to the plants: a certain amount of nitrates is washed away by water or is denitrified. This happens when conditions in the soil become favourable for the development of denitrifying bacteria. These take the oxygen necessary for their metabolism from nitrates, thus reducing these salts. Owing to loss of oxygen through the action of the bacteria, nitrates first become nitrites and then decompose further with liberation of gaseous

nitrogen into the air. The action of denitrifying bacteria is injurious to agriculture as it robs the soil of part of its nitrogen.

The sources of nitrogen supply we have mentioned cannot make good the enormous wastage of nitrogen and its expenditure for plants' consumption.

Where do plants obtain additional nitrogen?

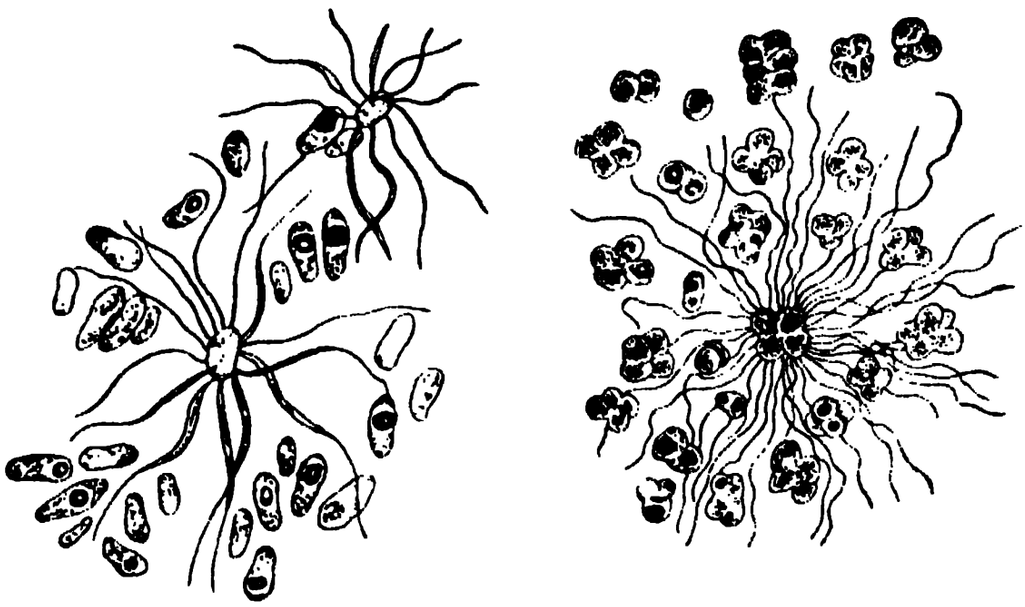

Fig. 26 Urea bacteria

Legume plants, such as pea, lupin, beans, grow very well on meagre soil containing little nitrogen and yield good crops. A soil in which legume plants grow does not lose its nitrogen, on the contrary, if such plants grow in a soil for only one year, its nitrogen content increases. Consequently, legume plants contribute to a higher soil productivity.

This property of legume plants was known to the ancient Chinese and Japanese who ploughed in these plants at the time of blooming, thus introducing into the soil nitrogen and organic substances.

Varro, an ancient Roman writer, recommended to manure a meagre field with lupin shoots instead of farm manure. Green manure is used to this day.

The ancient Romans knew that if beans were sown after wheat the productivity of the soil would be higher.

But the reason for this was unknown. Many scientists worked on the solution of this problem and finally it was discovered that the higher soil productivity was due to

Fig 27 Nodular bacteria on the roots of legume plants

the small nodular projections on the roots of legume plants (Fig. 27). The Russian scientist M. Voronin discovered in 1895 that the small nodules were full of bacteria which were called nodular bacteria.

Investigations by other scientists have shown that the nodular bacteria developing in the roots of leguminous plants fix the nitrogen they receive directly from the inexhaustible store of it—the atmosphere.

Here again we have an example of symbiosis. The legume plants receive nitrogen formed in the bodies of the nodular bacteria while the latter extract from the plant roots the carbohydrates they need as nutriment.

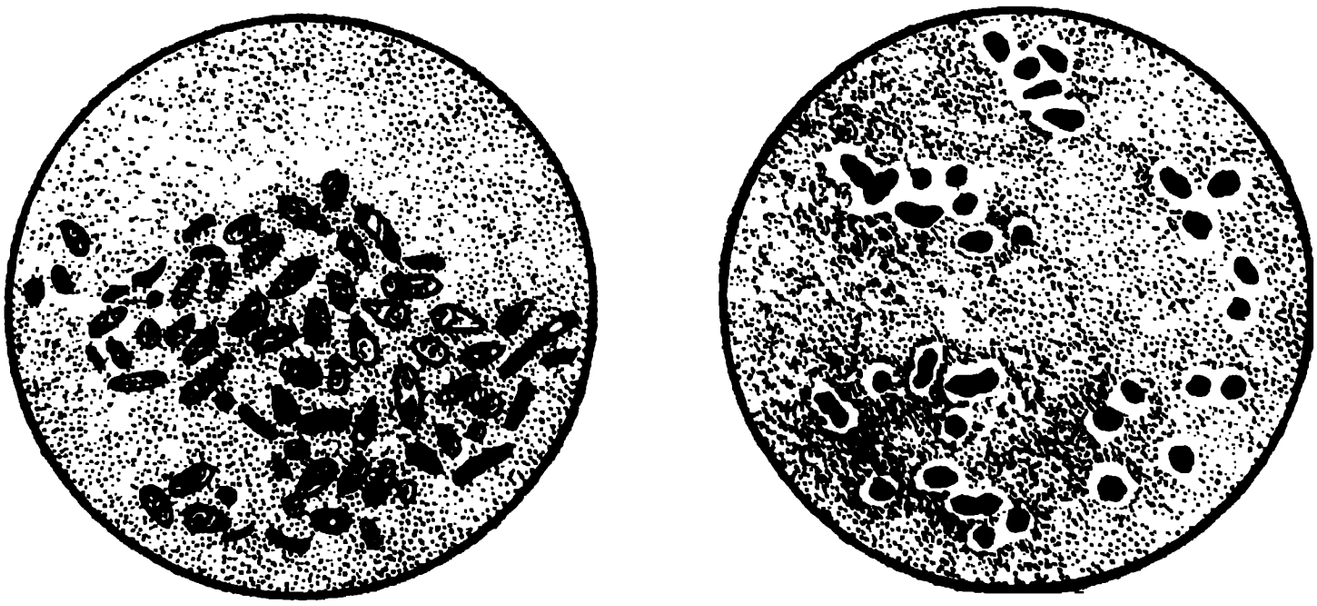

Fig. 28 Nitrogen-fixing bacteria, *1—Clostridium pasteurianum* and *2—Azotobacter*

At the same time as Voronin, another Russian scientist, S. Vinogradsky, discovered one more species of bacteria living in soil and fixing atmospheric nitrogen. This organism which Vinogradsky named in honour of Pasteur *Clostridium pasteurianum* (Fig. 28, *1*) and which can live under absolutely anaerobic conditions (see Chapter II) causes butyric acid fermentation.

Another micro-organism discovered in 1901 by the Dutch scientist Beijerinck and named Azotobacter assimilates atmospheric nitrogen in still greater quantities (Fig. 28, *2*).

There are other micro-organisms capable of assimilating nitrogen from the atmosphere but in smaller quantities than do the *Clostridium pasteurianum* and the Azotobacter.

The important role played by nitrogen-fixing bacteria can be seen from the fact that during the warm seasons they assimilate from 50 to 250 kilogrammes* of nitrogen per hectare of soil (D. Pryanishnikov).

Unfortunately, these bacteria do not occur in all soils and some of them possess but low activity. That is why it was decided to prepare artificial fertilizers consisting of nitrogen-fixing bacteria. And indeed, if colonies of nodular bacteria are introduced into a soil better crops are obtained, for in this way the number of nodules on the roots of legume plants is highly increased.

Nitragin, a preparation of different strains of nodular bacteria, was made and in 1934-36 experiments in its application as fertilizer were undertaken on a large scale. The average increase in crops was 20 to 30 per cent.

Experiments conducted at the Institute of Microbiology under the Lenin Academy of Agricultural Sciences of the U.S.S.R. showed that harvests of some legume plants sown in nitragin-fertilized soils increased more than 50 per cent. When other fertilizers containing phosphorus, potassium and calcium were added, the increase was more than twofold.

The application of nitragin results in better yields not of legume plants alone but also of other crops such as wheat and rye sown for two or three years after the legumes.

With each successive year nitragin was applied in ever increasing quantities. While in 1934 it was used in the U.S.S.R. to fertilize 49,400 acres, in the next two years the area manured increased more than 12 times and in 1940 there was more than a hundredfold increase. At present nitragin is used in still greater quantities.

The Russian scientist S. Kostychev suggested the manufacture of azotogen or azotobacterin from Azoto-

* I.e., 110-550 lbs.

bacter and its use on fields of wheat, rye, oats, barley, potatoes and sugar beets. The application of azotogen brings about an average increase of crops from 10 to 15 per cent.

A hectare of soil contains from 5 to 6 tons of phosphorus. But it forms part of organic compounds or of poorly soluble compounds, i.e., of substances which plants cannot use, and therefore its presence cannot influence the crop yield.

Soviet scientist A. Menkina has discovered a group of bacteria which decompose the organic phosphorus compounds found in the soil and accumulate phosphoric acid necessary for plant growth.

This group of bacteria was used to prepare a new fertilizer—phosphorobacterin.

The introduction of phosphorobacterin into the soil raised the yield of oats, wheat and other crops from 15 to 30 per cent and more. But bacterial preparations prove effective only if favourable conditions prevail in the soil for the life of the bacteria. Further research in this field is still under way.

Since bacterial fertilizers are effective and cheap, a number of factories have been built in the U.S.S.R. producing nitragin, azotogen, phosphorobacterin and other preparations.

The analysis of soils has shown them to contain large amounts of potassium which is also necessary for plant nutrition. A layer of soil one metre thick over a hectare of land contains as much as 350 tons of potassium. This quantity would suffice to feed plants for thousands of years. But soil potassium forms part of complex salts—aluminosilicates—from which plants cannot extract potassium. That is why potassium is introduced into the soil as a fertilizer.

Recently Soviet scientists discovered the presence in soil of bacteria capable of decomposing aluminosilicates and reducing potassium to a plant-available form. Today

the problem of practical utilization of the bacteria is being closely studied. Experiments have shown that if seeds or soil are treated with the silicate bacteria before sowing, the quantity of potassium assimilated by the plants is greatly increased and higher yields are obtained.

Carbon forms part not only of proteins but of all non-nitric organic substances. Consequently, plants need carbon in order to grow and reproduce.

We know that all plants whose leaves and stalks are green owing to the presence of chlorophyll are capable of assimilating carbon from atmospheric carbon dioxide. Plants assimilate carbon dioxide also through the roots. Animals receive the carbon required for their subsistence from the plants they eat. Man receives it from vegetable and animal food. The only source of carbon known to us at present is atmospheric air.

The carbon-dioxide content of air is very low, making up 0.03 per cent of the total volume of atmosphere. But this percentage never decreases and remains constant.

It would be interesting to know the way in which the vast quantity of carbon consumed as plant food is compensated. Humans and animals exhale carbon dioxide in breathing, i.e., return to the atmosphere a certain quantity of carbon. A considerable amount of carbon dioxide is returned to the atmosphere through the burning of coal, oil, peat, and other fuels at industrial establishments and by transport. A sizable quantity of carbon dioxide is introduced into the atmosphere during volcanic eruptions. But all this cannot compensate the consumption of carbon dioxide by plants.

Let us recall that in the process of decomposition of animal and vegetable remains by micro-organisms various gases are produced, including large quantities of carbon dioxide. The activity of microbes releases more than 1,652,000 gallons of carbon dioxide per hectare, and that is the main source of atmospheric carbon.

Like man and animals, plants, too, need additional vital (biotic) elements—vitamins and vitamin-like substances. Plants are able to synthesize most of the biotics themselves, but some of them, for instance, vitamin B_{12}, they receive in a pure state from the soil where it is produced by certain actinomycetes; vitamin B_2 is chiefly produced by Azotobacter.

The biotics necessary for the good growth of plants are not found in adequate quantities everywhere and the practice now is to feed them to plants artificially. Thus greater harvests are obtained of fruits, tomatoes, etc., if the flowering plants are sprinkled with heteroauxin, a biotic which, in certain conditions, increases the yields.

The scientifically established phenomenon of antagonism between the various species of micro-organisms inhabiting the soil manifests itself in the vital products of one species, particularly fungi, being harmful to others. As a result of this harmful action, some microbes cease to reproduce and perish.

Since one species of microbes destroys others, the vital products of the former are called antibiotics.* These play an important role in the life of plants. Applied in small quantities, some antibiotics stimulate the development of plants while a number of antibiotics protect plants from infectious diseases. By extracting antibiotics from certain antagonist micro-organisms we obtain effective weapons against numerous infectious diseases of plants.

We have just described the beneficent effect produced on plants by the invisible organisms populating their roots. But root micro-organisms cohabit with plants only when this symbiosis is useful for themselves. If such is not the case friends may become enemies. When, for instance, nodular bacteria do not receive enough non-nitric substances, they turn into parasites. The same

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* Antibiotics are discussed at greater length in Chapter XI.

occurs under adverse conditions of growth of the plants. L. Sudakova has observed clover crops lowered when nitragin was introduced under conditions of insufficient light.

* * *

It is an accepted idea today that soil micro-organisms are a very important factor in enhancing soil productivity. Their effect on crops, however, largely depends on cultural practices. It is therefore clear that agriculturists must be able to determine the best methods to be used in given local conditions.

T. Maltsev, a collective-farm scientist, field expert at the Lenin Collective Farm and director of the Shadrinsk Experimental Station, Kurgan Region, has introduced new methods of tillage for annual crops on his farm. He gave up ploughing the soil with the turning of the upper layer every year and proved that every successive annual crop far from exhausting the soil serves to progressively heighten its productivity. Maltsev's method as applied on the Lenin Farm has yielded excellent results.

His method of tillage creates favourable conditions for the activity of micro-organisms of the soil, because it forms what is known as "active humus," making for a good structure of the soil.

Maltsev's method is being tested under various conditions with a view to determining its applicability to other parts of the country.

* * *

The micro-organisms we have been dealing with so far are essential for normal plant growth, for raising soil productivity and the yields of cereal and other crops. But there are many harmful organisms known as phytopathogenic* microbes which cause various infectious plant diseases.

* From Greek *phyton*—plant, *pathogenic*—causing disease.

A. Yachevsky studied the diseases of some 300 plant species and found them subject to nearly 600 various diseases, of which 280 can lower the harvest or destroy a crop altogether. We shall describe a few of these diseases.

Some diseases of cereal crops, such as smut, are very detrimental to harvests. Smut is caused by a parasitic microscopic fungus developing in the ears of oats, wheat and rye or in corn-cobs, which destroys the grains and produces an enormous quantity of spores giving rise to new generations of the fungus. The spores which look like black powder are carried by the wind and infect healthy plants.

Ergot is another fungus which develops in place of grains, mostly in rye ears, in the form of dark purple horns (Fig. 29). Ergot is poisonous and if its admixture in rye bread is considerable, such bread may cause a dangerous disease in man. The disease manifests itself in giddiness, convulsions and is often fatal. In olden times villagers called the disease "evil convulsions." Nowadays, ergot in rye is very rare in the U.S.S.R., thanks to proper agrotechnical measures—treatment of grain seeds and correct crop rotation.

Ergot, however, does not cause only harm: it is used as a haemostatic drug, enhancing blood coagulation.

In early spring when the snow begins to melt the shoots in winter-sown fields are sometimes covered with a greyish-red film. If the soil is very damp the lower part of the stalks rots and the shoots stick to the ground.

This disease, snow mould, is caused by *Fusarium nivale*, a microscopic fungus, which affects the shoots of winter wheat and rye and is very injurious, particularly when the snow thaws slowly.

The *Fusarium graminearum*, another fungus, affects the grains of rye, barley, wheat and oats and makes them poisonous. Bread made of flour from grain affected with the fungus is known as "intoxicating bread" or the

Mi-chung of the Chinese. After eating such bread a man feels weak, starts vomiting and acts like a drunken person. In the thirties of the present century large areas under crops in North America were affected with this fungus. The grain poisoned with fusariosis was exported to Germany where it caused mass disease of humans and animals. "Intoxicating bread" is sometimes to be met with in the Far East, less frequently in the central regions of the European part of the U.S.S.R. and in Western Europe.

People who have eaten bread prepared from grain left over for the winter under snow and collected in the spring, contract a disease whose symptoms are like those of food-poisoning.

The investigations of Soviet scientists, and especially of A. Sarkisov and his co-workers, have shown that during the winter a poisonous fungus *Fusarium sporotrichioides* develops in grain left under the snow. The disease caused by this fungus is called alimentary-toxic aleukia.* Certain micro-organisms cause diseases of rye, wheat and other cereals known as rust. These fungous infections occur pretty often.

Rust affects also beet, French beans, and other legume plants. It destroys the leaves and prevents fruits and seeds from forming.

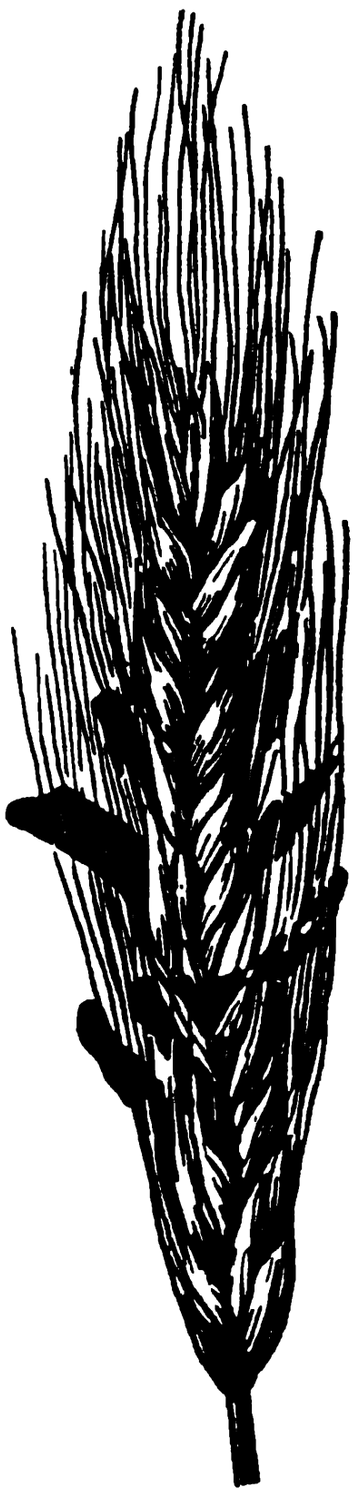

Fig. 29 Ergot in a rye ear

* A. K. Sarkisov, *Mycotoxicosis*, Selkhozgiz Publishing House, 1954.

One of the many potato diseases—mildew (or late blight)—is caused by a fungus *Phytophthora infestans*; although this is a frequent disease, the fungus cannot develop and the disease does not spread in a dry summer, but if the end of summer and autumn are rainy, the disease can seriously jeopardize the potato crop. It affects both the leaves and the tubers. First dark spots appear on the leaves and stalks, then all the green parts blacken and rot away. Then dark-brown hollowed-out spots appear on the tubers, later these spots develop into damp rot which destroys the whole tuber. The Phytophthora usually affects early-ripening potato varieties.

Fig. 30 Potato plant affected with blackleg (after V. Izrailsky)

Some fungi cause various flax diseases, e.g., fusariose, manifested in the yellowing and wilting of the plant, and anthracnose destroying almost all flax shoots. Flax is also subject to mildew, rust, and other fungous diseases.

Fungous diseases are specific, in the sense that a fungus infesting one species of cereals, e.g. maize, does not attack another, say oats.

But it is not microscopic fungi alone that attack plants: a large number of infectious plant diseases are caused by bacteria.

Almost all plants are liable in certain conditions to be affected by one or another bacterial infectious disease.

The *Bacterium malvacearum*, for instance, infects the cotton plant, causing the appearance of angular black spots on the leaves. The leaves shrink and fall off, slime appears on the bolls which, subsequently, rot away. This cotton disease occurring in all parts of the world is successfully combated in the U.S.S.R.

Another bacterium—the *Erwinia carotovorum*—causes soft rot in many vegetables, such as carrot, cucumber, onion, lettuce, cabbage, tomato, melon and others. This disease is widespread in the U.S.A., Canada, Europe and other countries.

Oranges and lemons have their specific bacterial diseases; there are bacteria which affect only tomato; the potato bacillus causes potato and pumpkin rot.

A common potato disease called blackleg causes the yellowing and wilting of the leaves, which do not attain normal size; the stems blacken and rot at the base, and often the plant is destroyed before any tubers appear. There were years when as many as 1,200,000 tons of potatoes were lost in the U.S.A. through this disease.

Sugar beet may be affected with scabs, cancer of the root and other diseases. In Fig. 31 we see beet affected with what is known as tuberculosis occasioned by the *Xanthomonas beticola.*

The *Bacterium viticola* is known to cause the wilting of unripe grapes.

There is also bacterial cancer* of tomato and many other diseases caused by bacteria.

We know of more than 200 plant diseases caused by viruses, as for instance, tobacco mosaic, the study of which helped D. Ivanovsky to discover filter-passing viruses (see Chapter I).

* Tumours on plants known as cancer have nothing in common with the disease of man.

Further study showed that filter-passers cause very many diseases. Tobacco alone is subject to eight types of the mosaic disease (Fig. 32), potato to five, maize to two and raspberries to three types. Mosaic attacks various vegetables, buckwheat, legume plants and flowers.

And this is not all: plants also have other diseases, caused by viruses, such as brown rot in peach, straw-

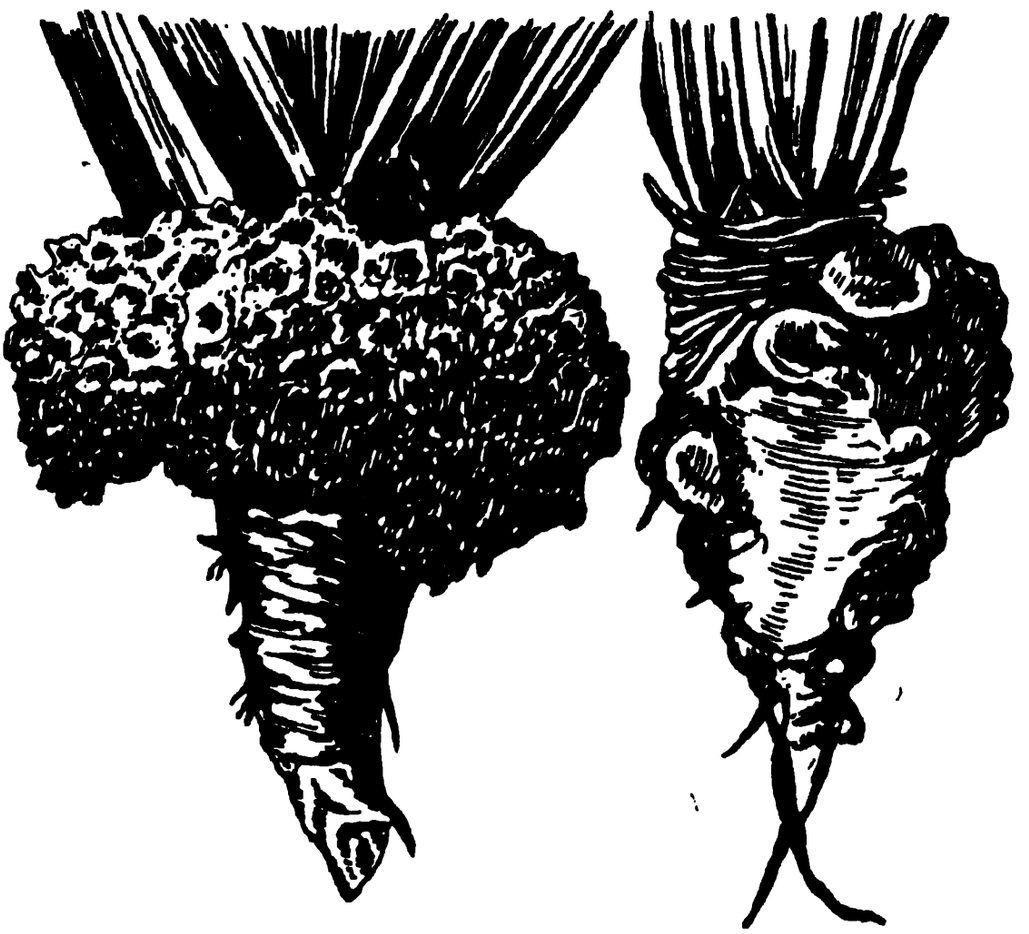

Fig. 31 Beetroots affected with tuberculosis (after V. Izrailsky)

berry, Chinese aster, wheat rosette (the latter disease attacks also peach and other fruit trees), potato leaf curl, sugar-cane diseases, etc.

The following data from the experience in the U.S.A. given by T. M. Rivers in *Filterable Viruses*, show the danger bacterial and virus diseases constitute to cultivated plants. In the State of Washington three-quarters of the sugar beet crop were destroyed by curly top in 1924.

Virus diseases are responsible for heavy losses in many of the leading potato-growing states. In some

parts of the country cucumber mosaic ruined the farmers. Sometimes crops of maize were completely destroyed by corn mosaic.

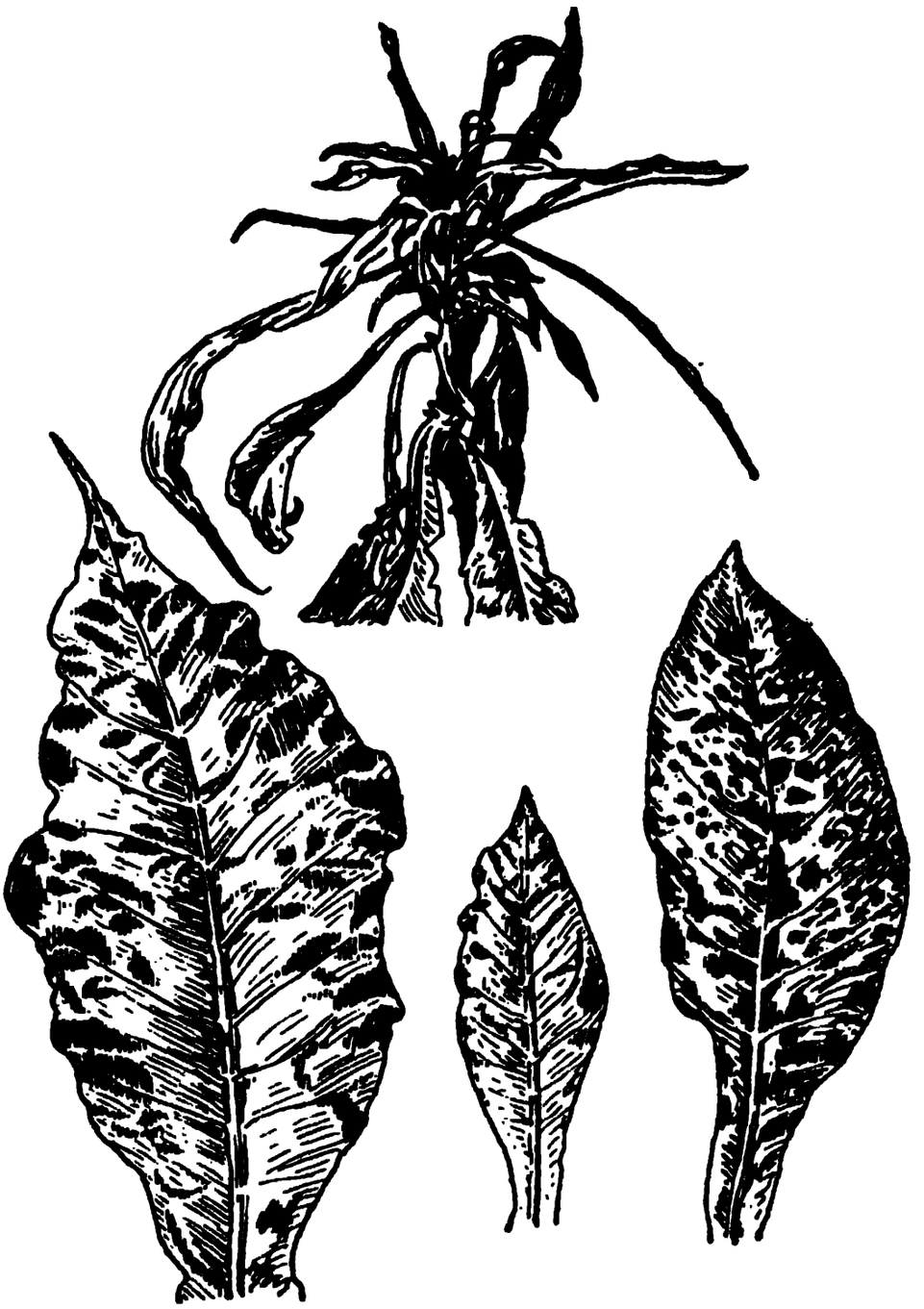

Fig. 32 Tobacco leaves affected with mosaic (after D. Ivanovsky)

There were years when gummosis, a bacterial disease of the cotton plant, destroyed up to 78 per cent of the crop, when calico destroyed 30 per cent of the tobacco crop and pea fire blight 25 per cent of the pea crop. Sometimes all French bean seedlings were destroyed by

bacteriosis. Bacteriosis, too, was responsible for the loss of 54,000,000 tons of wheat and from 25 to 100 per cent of the oat crop. Bacterial fire blight of apples and pears brought annually a loss of 10,000,000 dollars in California, while banana bacteriosis became so widespread that the cultivation of this plant was discontinued on an area of 49,400 acres.*

The question that naturally arises is how can plants exist if they are in constant danger of so many diseases?

First, not all plant species are equally susceptible to infectious diseases. Second, an infectious disease develops in plants only when there are favourable conditions for the causative organisms, the phytopathogenic microbes. Third, soil has been discovered to contain a number of antagonist micro-organisms capable of destroying or inhibiting the growth of phytopathogenic microbes, and thus protecting plants from disease. There are, for instance, bacteria oppressing microbes causing flax diseases.

Plant roots, especially the roots of legume plants, often are populated by bacteria causing the lysis of harmful fungi. The presence of such antagonists serves as protection against pernicious fungi.

Fusariosis, a disease caused by the fusarium fungus, affects flax, cereal crops, tea bushes, pine seedlings, and many other plant species. But antagonist bacteria have recently been discovered which are an effective protection against fusariosis.

Good results have been obtained from the application of a species of actinomycete against the fusarium wilt of cotton. There are descriptions of effective use of antagonist microbes against potato scab, some fungous diseases of cucumber, cabbage and other vegetables. Under natural conditions this happens when antagonist microbes (bacteria, actinomycetes or fungi) develop in the

* *Bacterial Diseases of Plants*, edited by V. Izrailsky, Selkhozgiz Publishing House, 1952.

same rhizosphere in which corresponding phytopathogenic organisms are active. The action of the antagonists greatly inhibits the growth and reproduction of the phytopathogens, and the surviving individuals become subject to lysis, i.e., dissolution. This phenomenon is often observed in crop rotation when one crop succeeds another. If in Central Asia, for example, cotton is grown in the same field for many years running, it is often affected with wilt, an infectious disease caused by the fungus *Verticillium dahliae*. But if alfalfa is sown in the cotton-sick field the phytopathogenic fungi will, in the course of a few years, be supplanted by the antagonist bacteria which breed in large quantities on the roots of alfalfa.

Fig. 33 Tobacco leaf affected with calico (after V. Izrailsky)

Numerous experiments in antagonist bacteria application are being carried out to protect plants from infectious diseases. Pure cultures of antagonists are bred in culture media and seeds are treated with them before sowing. Sometimes the cultures are worked into the soil with water. Another method of treatment proposed by N. Krasilnikov consists in dipping the roots of seedlings in the suspension of an antagonist culture before planting. All these methods protect plants from parasitic microbes.

The wilting of apricot and peach trees in Armenia is caused by the *Bacterium armeniaca*. First the leaves of the affected plant dry up, then the branches, and finally

the tree dies altogether. R. Mirzabekyan applied an antibiotic extracted from an actinomyces. She moistened with the antibiotic the leaves of the affected tree, or injected it into the trunk.

Antibiotics have been proved effective in treating lemon and tangerine fruits affected with bacterial necrosis.

Cotton gummosis is caused by the *Pseudomonas malvacearum*, a bacterium which often finds its way into cotton seeds. In 1951 S. Askarova treated cotton seeds with an antibiotic which is capable of entering the seeds and killing the gummosis germ without causing any harm to the seeds or affecting their germinative power. Askarova's experiments showed that the treatment of cotton seeds with the antibiotic lowers the incidence of disease to 0.2 per cent.

Actidione is an antibiotic used against the mildew of legume plants. Two days after sprinkling the fungous mycelium on the leaves is destroyed and the white deposit vanishes.

Grizeofulvin protects lettuce from the infectious fungus *Botrytis cinerea* and tomato from the *Alternaria solani*.

Some scientists hold that antagonist micro-organisms developing in soil excrete their antibiotic substances into the soil, then plants absorb them and thus become immune to infectious diseases. Consequently, the inborn immunity of plants is due not only to the presence of phytoncides (bacteriolytic substances) produced by the plants themselves within their living tissues, but also to the penetration of antibiotics into plant tissues.

The observations and experiments described here open up a way of combating infectious diseases of plants and point the direction in which the study of plant immunity should proceed. Since weakened plants are more susceptible to disease, it is clear that good cultural practices are an effective weapon against injurious microbes.

Chapter IV

THE EARTH'S HISTORY AND MICRO-ORGANISMS

When speaking of the history of our planet the first question that arises is: How old is the earth?

According to the Bible the earth is 7,462 years old. From ancient Chinese annals it would seem that it is more than 3,000,000 years old, while Japanese legends say that the Japanese Isles were populated several million years ago.

Geological research by exact physico-chemical methods based on the radioactivity of chemical elements furnishes strong proof that life appeared on the earth about 1,000,000,000 years ago.

The earth's history has been studied from the annals Nature had itself created.

The crust of the earth consists of superimposed layers. The difference in the strata has enabled geologists to divide its history into eras which are subdivided into periods, and the latter into epochs or ages.

The earliest era in the earth's history is known as Azoic, i.e., lifeless, for no trace of either plant or animal life has ever been found in Azoic rocks. The Azoic era must have continued for about 700 million years. The next era is called Proterozoic or Eozoic, which means the dawn of life, for at that time the first signs of life appeared on earth. This era lasted for about 500 million years. The following, Palaeozoic, era, which continued for about 350 million years, is subdivided into several periods: Cambrian, Silurian, Devonian, Carboniferous and Per-

mian. Then came about 160 million years of the Mesozoic era which includes the Trias, Jurassic and Crestaceous periods. The Mesozoic era was followed by the Kainozoic era, subdivided into the Tertiary period which lasted about 59 million years, and the Quaternary period we are living in now, which is about a million years old.

A very important and interesting page in the history of our planet tells of the formation of the fossil fuels, i.e., anthracite, coal, lignite, peat, shales and petroleum.

What is the origin of the fossil fuels?

In the roofs of coal seams, i.e., in the limestone, sandstone and mudstone covering coal seams, traces of leaves, cones, branches, tree-bark and even seeds have been discovered. The geologists call these traces impressions. Vegetable remains have also been found in rocks forming the floor of coal seams.

Big fossil forests—upright tree-trunks with roots stretching from them—known as *stigmaria*, have been discovered, which in the course of millions of years were transformed into coal.

Microscopic examinations of thin sections of coal and peat have revealed fragments of cellulose, particles of wood cells and of other resistant parts of plants. These findings show that coal and peat are the transformed products of vegetable remains.

But how were plants transformed into coal?

Beneath the carpet of fallen leaves, twigs and pine-needles in a forest there is always a layer of humus, the product of decay of vegetable matter by the action of putrefactive and other micro-organisms. Humus consists of a mixture of humic and ulmic substances.

Scientists have established that anthracite, coal, lignite and peat have been formed from humus, therefore these fuels are called humic.

Peat consists of a mass of semi-decayed vegetable remains held together by a small quantity of humic substances. Coals contain a small amount of vegetable mat-

ter consisting of the more resisting parts of plants but in the main they are made up of humic substances.

If we look deeper into the origin of peat and coals we shall see that here, too, great differences prevail. Anthracite was formed millions of years ago. Psilophytes, the oldest cryptoganic land plants resembling seaweeds or lycopodium, were the mother substance of anthracite.

The formation of the greater part of the coal deposits began in the Carboniferous period from dead and decomposed fern-like plants, such as the lepidodendrons, sigillaria, calamites, lycopodium and mare's tail.

Lignites were formed at a much later time in the subtropical and moderately warm climate, and peat was formed in the present period of moderately cold climates from coniferous residue, angiospermous plants and mosses.

Thus we see that whereas anthracite and coals were formed more than 300 million years ago, lignites are only a few score million years old, and that peat began to form a few hundred thousand years ago, the process continuing to this day.

As has been stated above, anthracite, coal and peat were formed from humus, and humus itself is the result of the action of microbes on vegetable matter. It may therefore be assumed that fossil fuels resulted from the activity of micro-organisms which decomposed vegetable remains of different geological periods, beginning with the most ancient.

Let us see how correct this hypothesis is.

Cigarette-holders, brooches and beads made of amber are familiar to everybody. Amber is fossilized resin of coniferous plants found in Tertiary strata. Flowers resembling daisies, butterflies and bees have been discovered in amber, and on the bodies of the flowers and insects myriads of mould and yeast fungi. Microbes were also found on the antennae of the bees and on the proboscis and antennae of the butterfly. All this can be seen

through amber as through glass. Everything has been preserved in a perfect state for millions of years, because the insects and microbes had been covered with resin which subsequently became fossilized.

Fossil remains of plants and especially of trees are often met in deposits of different geological periods, including the most ancient. In these remains spores and parts of microscopic fungi have been discovered. Remains of microbes have also been found in lime shale containing coal. Numerous plant petrifactions in coal seams show traces of cells attacked and more or less destroyed by microbes. In places where the plant tissue is broken, rods and cocci have been observed in chains, clusters or scattered, greatly resembling bacteria and cocci of today.

It seems obvious that these micro-organisms were petrified along with the remains of the plants they were decomposing.

The Moscow Coal Basin is said to produce "leafy" coal, which can readily be separated into thin layers. If we take a thin leaf of the coal and treat it with ammonia, part of it will dissolve and we shall have an almost transparent cuticle of a plant. A microscopic examination of the cuticle will reveal small hollowings on the surface in which one or two minute grains are deposited. These are cocci, very much like the modern form in both shape and size.

A very large number of petrified vegetable remains containing microbes, the agents of their decomposition, have been found in old layers of the earth's crust.

The question arises, however, whether microbes could live in the conditions of fossil fuel formation.

It has been established that the formation of these fuels (even at a depth of 1,000 metres) occurred at temperatures suitable for the life of micro-organisms. The pressure, which at that depth was 250 atmospheres, could not have interfered with the life of microbes, for they are capable of enduring pressures of 12,000 atmospheres and more.

In this way myriads of micro-organisms decomposed dead plants from which in the course of thousands and millions of years anthracite, coal, lignite and peat were formed. And perhaps, if there had not been any microbes we would not have any coal and peat today.

Petroleum, another fossil fuel, also plays a very important role in the national economy. Petroleum after refining yields benzine, paraffin (kerosene), lubricating oils and over 120 other very essential petroleum derivatives. Mazout, which is also derived from petroleum, is an excellent fuel for furnaces and engines.

Whereas the remains of land plants were the initial material for the formation of humus and humic substances (coal, peat, etc.), bituminous substances are the result of the decomposition of water and (partly) land plants. The humic substances have turned into coals, coal resins containing carbolic acid, naphthalene and other compounds, and also coal gas. But petroleum and its products such as benzine, paraffin, and various oils, belong to the bituminous group.

Academician N. Gubkin and other Soviet scientists hold that petroleum was formed in ancient times from vegetable and animal remains.

How did all this occur?

The investigations carried out by T. Ginzburg-Karagicheva and others show that vegetable and animal remains are decomposed by groups of various micro-organisms producing hydrocarbons which form part of petroleum.

Petroleum is found in all geological periods. This indicates that conditions for its formation were favourable in quite a number of geological epochs, including the present. This is borne out by Soviet scientists who have discovered "oil" micro-organisms at very great depths in petroleum strata. Consequently, microbiological processes conducive to the formation of petroleum are still taking place.

Before petroleum can be extracted it has to be found. The search for deposits of minerals is called prospecting. Scientists have established the existence of such microbes which utilize petroleum gases—methane, ethane and propane—even if they escape from the earth's depths in such minutest quantities that they cannot be detected by chemical means. This prompted Soviet scientists V. Butkevich and G. Mogilevsky in collaboration with a group of microbiologists to use microbes as indicators of new oil deposits. Instead of looking for petroleum and petroleum gases, microscopic doses of which are very difficult to detect, they decided to use the microbes which multiply in the soil and subsoil in the presence of negligible amounts of the gases. In this way the existence in a soil or subsoil of certain "oil" organisms points out a possible source of petroleum.

* * *

Ignis fatuus, or will-o'-the-wisp is a phenomenon familiar to all, if not from personal experience, then from books or hearsay.

We know that plant and animal matter is decomposed by a variety of microbes. This process is accompanied by the production and accumulation of various gases, including burning gases, such as methane, hydrogen and others. Methane, the product of anaerobic decomposition of cellulose, is called marsh gas, because it sometimes escapes to the surface of a swamp in different places and, coming in contact with the air, ignites. That is how the phenomenon got its present name.

The activity of microbes within the earth has resulted in enormous accumulations of natural gases which today are widely used in the home and for industrial fuels, as well as for processing.

The above clearly illustrates the great role microbes have played in the earth's history. The impressions of various fossil microbes and the plant matter petrified

while the microbes were active decomposing it are proof of the fact that the earth was populated by different types of micro-organisms in the most ancient times. It may well be that these invisible organisms were the oldest living beings on our planet.

* * *

The activity of micro-organisms has resulted not in the production of fossil fuels alone.

For thousands of years the Atacama Desert in Chile and the Pampa Negra in Peru have given refuge to countless numbers of sea fowl, which nested there, hatched their young, lived and died. A mass of their excrement and dead accumulated, and as rains are very rare in these places, their remains were not washed away and large deposits of the birds' corpses and excrement known as guano, were formed.

Acted upon by putrefactive bacteria, guano was decomposed with formation of ammonia and nitrifying bacteria converted ammonia first into nitrites and then into nitrates, such as saltpetre. For thousands of years it accumulated in these deserts forming extensive beds of the mineral which is used in a number of countries as fertilizer, and also for industrial purposes. Saltpetre deposits of similar origin but of less magnitude have been discovered in Egypt, India, and other countries.

Another hypothesis is that saltpetre was formed out of seaweeds collecting in immense quantities on ocean shores and subsequently decomposed. Both theories acknowledge the agency of micro-organisms, for no matter whether the initial material is guano or seaweeds, some microbes decompose it and other convert the nitrite compounds into saltpetre.

* * *

A visitor passing the famous estuaries in Odessa is struck by a smell of rotten eggs, i.e., of hydrogen sul-

phide, which reaches him even from a distance. Where does it come from?

There are bacteria reducing sulphates by taking away their oxygen and liberating hydrogen sulphide. Another source of this gas is decomposition by putrefactive bacteria of vegetable and animal residue accumulated at the bottom of sea estuaries, salt lakes, inland seas, and other reservoirs with stagnant water. That is why the water in Odessa estuaries, Tambukan lakes near Pyatigorsk, and other similar pools is filled with hydrogen sulphide. The waters of the Black Sea contain large quantities of this gas at a depth of 660 feet and more, the result of the activity of bacteria living at the bottom of the sea. The waters of the Arctic and the Atlantic oceans, too, contain hydrogen sulphide.

Besides bacteria reducing sulphates and producing hydrogen sulphide, there are bacteria known as sulphur bacteria, oxidizing the hydrogen sulphide produced at the bottom of lakes, estuaries and seas.

Although many varieties of sulphur bacteria have been discovered, all come under one of the two categories: colourless and coloured. Colourless sulphur bacteria require atmospheric oxygen for their metabolism, i.e., they are aerobic. The coloured sulphur bacteria, sometimes called purple, although their colouring may be anything, from brick-red to pink or even green, are anaerobes. Sulphur bacteria differ from all other bacterium species by their size, some of them, as for instance the *Beggiatoa mirabilis*, being 1 cm long and 45 μ wide, so that they can be seen with the naked eye. These are real bacterial giants (Fig. 34). At the oxidation of hydrogen sulphide by sulphur bacteria grains of pure sulphur are produced which accumulate in their bodies as well as in their environment.

Therefore, not all deposits of native crystalline sulphur are of volcanic origin; in localities where there are no volcanoes sulphur accumulates as a result of bacterial

activity. Large masses of dead bacteria decompose, releasing sulphur which accumulates, forming deposits of this mineral.

The rich deposits of sulphur discovered in the Caucasus, Central Asia, North America, and other countries, are believed to be of bacterial origin.

Whenever the water in a reservoir contains ferrous salts (which is almost always the case) it invariably contains iron bacteria.

Iron bacteria consist of several cells joined together in the form of threads, sometimes branching off. In most iron bacteria, the threads are encased in mucous capsules (Fig. 35, left); some bacteria proliferate by forming twisted mucous ribbons, like interlaced hair-pins with the bodies of the iron bacteria at the ends (Fig. 35, right). The bacteria use their threads to attach themselves to stones, grains of sand at the bottom of a river or lake, to stalks or leaves of water plants. In the process of metabolism iron bacteria oxidize great quantities of carbonate of iron. The resulting ferric hydroxide impregnates the mucous capsules of the bacteria. Bacteria are often found with iron-impregnated capsules weighing three times as much as the bacteria themselves. As they die off, the accumulated iron is deposited in layers of varying thickness.

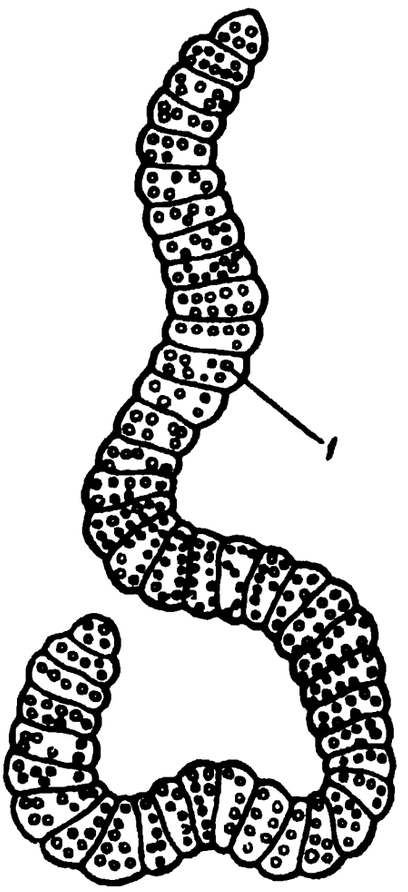

Fig. 34 The giant sulphur bacterium *Beggiatoa mirabilis* filled with sulphur grains (*1*)

Large quantities of oxygen and carbonate of iron are dissolved in the water of many reservoirs. From these, iron bacteria produce ferric hydroxide. Myriads of bacteria accumulate and deposit iron on stones and other objects, as well as at the bottom of the reservoirs. The

mass of iron, at first loose, settles on stalks and roots of seaweeds and other water and herbaceous plants which die off and are then decomposed by saprophytic bacteria. The iron deposit becomes a reddish mass honeycombed with small channels left by the stalks and roots. That is how deposits of brown hematite are formed.

Other iron ores, known as bog and lake ores, are the product of similar bacterial activity. In localities abound-

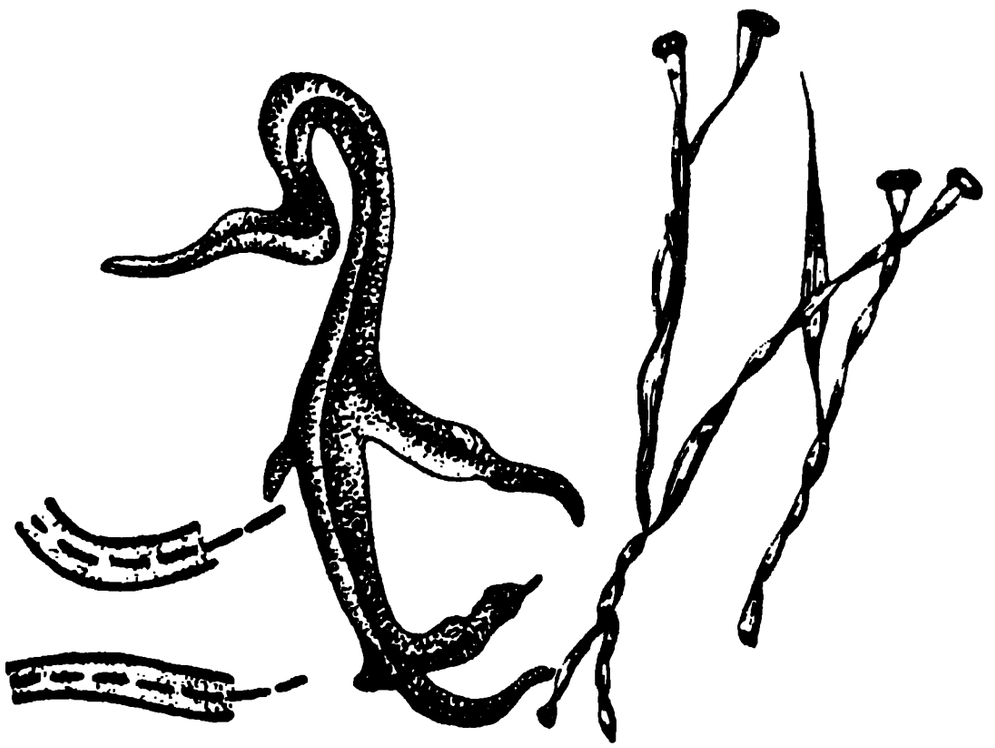

Fig. 35 Iron bacteria

ing in rivers, lakes and swamps bacterial ores are deposited in hundreds of thousands of tons every year.

Besides iron, the water in some reservoirs contains manganese. Since iron bacteria can oxidize carbonate of manganese just as they do ferrous salts, their activity results in deposits of manganese ore. Iron and manganese occur in minute quantities in various rocks forming beds of rivers, bottoms and shores of lakes and swamps. Iron bacteria collect as it were the minute grains of iron and manganese and in the course of centuries build up deposits of these ores.

Thus we see that iron bacteria are an important factor in the formation of considerable deposits of iron and manganese.

The Odessa estuaries and some other inland reservoirs are famous for their curative muds, which are used in treating rheumatism and other diseases. An analysis of these muds, i.e., of black or greyish-black silt, has shown that they contain ferrous sulphide, the product of the interaction of hydrogen sulphide and ferric hydroxide usually occurring in large quantities in such reservoirs. And now we know that both hydrogen sulphide and ferric hydroxide are produced by sulphur and iron bacteria.

Besides ferrous sulphide, curative muds are frequently found to contain pyrite. A comparison of the formula of ferrous sulphide FeS and of pyrite FeS_2 shows that pyrite has twice as much sulphur. In the opinion of some Soviet scientists (Tauson), the transformation of ferrous sulphide into pyrite is the work of the microbes which form sulphur and hydrogen sulphide. An excess of one of these elements brings about the formation of pyrite.

* * *

Everyone is familiar with lime which is produced from limestone, one of the commonest rocks. Layers of limestone hundreds and thousands of metres thick are frequently found in the mountains. Limestone occurs also in valleys and in slightly elevated localities. In the U.S.S.R. great quantities of limestone occur around Moscow, Kazan, in the Zhiguli Hills, and other places. Reefs in seas and oceans, too, are of limestone.

What is its origin?

It has been established that limestone consists partly of the remains of various invertebrate animals, such as their skeletons, tests, shells, etc. And there are bacteria which produce carbonate of calcium, i.e., limestone or

chalkstone. These bacteria are widespread in Nature. Observations show that many different microbes produce lime.

Bacteria liberate carbonate of calcium in hot springs as well as in brackish and sweet waters. According to a hypothesis, this is the way in which the most ancient, pre-Cambrian, deposits of limestone were formed.

The Bahamas, off the coast of Florida, consist wholly of limestone. The sea round them abounds in bacteria capable of depositing carbonate of calcium. Investigations have suggested the idea that the Bahamas were formed by bacteria in the course of millions of years.*

* * *

But microbes do not only build, they also destroy.

We know that in the process of metabolism nitro-bacteria produce nitrites and nitrates. Nitric acid easily dissolves carbonate of calcium, the main component of limestone. In this way the surface layer of limestones can be destroyed by microbes. Since nitro-bacteria are very widespread, the destruction of rocks due to their agency (known as weathering) can be observed in many parts of the world.

Besides nitro-bacteria, other bacterial species are responsible for the weathering of rocks. We know of bacteria producing hydrogen sulphide. Sulphur bacteria obtain energy by oxidizing hydrogen sulphide and in this way producing sulphuric acid. This acid can dissolve various inorganic compounds, including limestone. That is why in localities where much sulphuric acid is produced, lime and other rocks are subjected to dissolution, i.e., intensive weathering.

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* Lime deposits of presumably bacterial origin have been discovered in Lake Sevan, Armenia.

But the weathering of rocks is not only due to the action of certain specific microbes, such as nitrifying, sulphur bacteria, etc.

All saprophytes liberate carbon dioxide in the process of metabolism. The wind can plant saprophytes on any granite or basalt crag, on the surface of which there is a tiny layer of organic matter brought there with the dust. By forming carbon dioxide, these organisms dissolve and destroy not only limestone but even granite and basalt.

Some micro-organisms destroy organic matter. Soviet microbiologist V. Tauson observed a peat bog in the Pamirs dotted with dark brown knolls. But many of the knolls were light grey, almost white; these were populated by myriads of most varied microbes. Tauson explained the different colour of these peat knolls by the decomposition of the humic matter due to bacterial activity.

Such humic substances as coal and lignite are also subject to decomposition by micro-organisms. In coal and lignite seams live bacteria have been discovered at depths between 130 and 3,300 feet.

Charcoal left after a bonfire in a forest gradually disappears. The same happens to the charred remains of trees after a forest fire. This is caused by the action of microbes which oxidize charcoal, converting it into gaseous carbon dioxide, which disperses in the air.

A diesel ship leaves large tracks of oil in its wake. Oil floats on the surface of rivers near oil-cracking plants and other enterprises.

If you watch the oil spots you will see them gradually disappear. An analysis has shown that these spots contain bacteria capable of decomposing petroleum in the presence of water. This is how water autopurification is performed by bacterial activity.

Microbes have been discovered that decompose paraffin wax, a component of petroleum. Other bacterial species decompose paraffin and produce naphthalan, a curative substance.*

Thus microbes, the first living organisms to appear on earth, were instrumental in forming fossil fuels. Microbes create iron and manganese ores, limestone and other rocks. They can even build up islands, thereby altering geography. But under certain conditions microbes can destroy rocks, change the composition of petroleum, etc. Negligible in size, but possessing immense aggregate energy, micro-organisms exert great and varied influence on the history of the earth.

* B. Isachenko, *The Gelogical Activity of Microbes*, *Nature*, No. 8, 1950.

Chapter V

MICROBES IN FOOD AND FODDER

WINE-MAKING

Wine-making dates back to the ancient times when it was found that if grape juice is allowed to stand, froth appears on the surface, a sediment settles on the bottom of the vessel as a result of fermentation, and the juice loses its sweetness and acquires intoxicating qualities.

From bas-reliefs preserved on the walls of Thebe, the capital of ancient Egypt, we see how wine was made in those days: grapes were picked, then crushed by foot in a trough, strained in sacks, and then steeped to obtain must.

When wine is made at home the strained grape juice (must) is subjected to fermentation, which is produced by wine yeast (yeast fungi), minute oval-shaped bodies.

How does wine yeast get into the must?

Investigations have shown that wine yeasts responsible for vinous fermentation collect on the skins of growing grapes.

Wine yeasts have been discovered inside the body of the Drosophylla, a small fly feeding on fruit. The Drosophylla settles on vines, pierces the skin of the grapes and lays its eggs, thus introducing yeast cells into them. When the grapes are crushed, the yeast cells penetrate into the must and cause its fermentation. Chance yeasts, however, cannot be relied upon to produce good wine, and today pure cultures of select strains of wine yeasts are used in wine-making, which ensures good quality of wine.

Modern wine manufacture, based on centuries-old experience and numerous investigations, is an extensive and important science.

Different wines are distinguished by their flavour, bouquet, strength, and other properties, which are determined by such factors as variety and quality of the grapes, their ripeness, sugar content, and so on. The quality of wine yeasts, and methods of preparing and preserving the wine are also very important.

Grapes are picked for wine at a time when their sugar content is 18 to 20 per cent and acidity is at its lowest. The stalks and seeds are separated and the grapes are crushed by special machinery, yielding from 90 to 95 per cent of juice. The juice, or rather, the must, is left to settle in vats for a period ranging from 12 to 24 hours.

To prevent fermentation at this stage, sulphurous acid, inhibiting the activity of micro-organisms, is added to the must. Fermentation can also be delayed, the must made to settle and the wine clarified by cooling it to about 25°F.

The settled must is then drawn into barrels containing "starters"—a certain quantity of select wine yeasts. By that time the sulphurous acid content of the must has been reduced to 20-30 milligrammes per litre, which allows the select yeasts to multiply and start fermentation while the vitality of "natural" yeasts and other micro-organisms introduced into the must together with the grapes continues to be inhibited.

During fermentation, under the influence of the ferments of the wine yeast fungi the must sugar decomposes and alcohol, carbon dioxide and other substances are formed. In this way new wine is obtained.

This process applies to the preparation of white wine from white grapes. In making red wines and some local brands, as for instance, Kakhetian (Georgia), the must is not allowed to settle, but is fermented together with the pulp, skins and seeds of the crushed grapes.

Fermented wine is then drawn into barrels; new red wine is darker and thicker but it is soon clarified by the tanning and astringent substances in it. Thus Kakhetian fermented in October is suitable for consumption in December.

In making dry wines the sugar of the must is allowed to ferment completely—that is why such wines are sour. Their alcohol content is from 9 to 12 per cent. A larger percentage would inhibit the yeast, and, consequently, delay the process of splitting dextrose and producing alcohol.

Like good dry wines, sour-sweet or sweet wines have a peculiar flavour and bouquet. These are made of special sorts of grapes, with a higher sugar content, which are picked after they have passed ripeness and lost some of their moisture through evaporation.

The must with a high sugar content is not fermented to the end, and the wine obtained from it contains a certain amount of sugar. That is why dessert wines are sweet. But there are other methods of increasing the sugar content of wines. The commonest is by the addition of alcohol to fermenting must, which inhibits the activity of the yeasts and, consequently, prevents further fermentation. In this way the alcohol content of dessert wines is brought up to 16-20 per cent.

PRODUCTION OF ALCOHOL

Rhazes, a celebrated Arabian physician who lived in the 9th-10th century A.D., gives the following recipe for producing liquor: "A simple method of making liquor: take some mysterious substance in a quantity thou choosest, knead it to obtain dough; then let it ferment one day and one night, and at last put it all into a still and have it distilled."

Some historians construe the "mysterious substance"

to mean wheat. Probably Rhazes meant malted wheat, germinated grains, because germination of seeds was then considered mysterious.

Men learned to produce alcohol in ancient times, after distillation had been discovered. Aristotle, who lived in the 4th century B.C., often mentions in his works that heated water becomes steam which can be cooled and converted back into water.

It is believed that alcohol was first obtained by distilling wine, and later, when malting was discovered, it was produced from wheat and other grains.

Alchemists attached great importance to alcohol, which they called quint-essence, i.e., the fifth element, the four other "essences" being earth, water, air and fire.

Although alcohol was known in ancient times, at first it was used only for medical purposes, the ancient physicians believing it to be a remedy against all diseases and to possess the rejuvenating and life-prolonging power. The miraculous properties attributed to alcohol, to say nothing of its intoxicating properties, may well have been the reason why it quickly spread in various countries.

In due time alcohol began to play a big part in medicine, chemistry and industry.

Today alcohol is produced from potatoes, rye, maize, molasses, cellulose sugar, and other substances. It can be distilled from apples, pears, beet, chicory, ferns, seaweeds, even from peat, straw, sawdust, and wood-shavings.

When alcohol is obtained from grain or potatoes, these are first sweetened with malt—germinated grains of barley, wheat or rye. Malt contains diastase or amylase—a ferment converting potato or cereal starch into maltose. Then special strains of the yeast fungus *Saccharomyces cerevisiae* split the maltose and produce alcohol. Vinous fermentation caused by alcoholic yeasts usually yields a wort with an alcohol content of 14-15 per cent. A

stronger alcohol is obtained by distilling fermented wort.

Large quantities of alcohol are required annually for the medical and pharmaceutic industries, as well as for production of synthetic rubber, and other goods. This necessitated the use of other raw materials, as for instance sulphite liquor, a by-product of the paper industry containing from 1 to 5 per cent sugar, for alcohol production.

Wine alcohol can be produced from wood: acted upon by sulphuric or hydrochloric acid, cellulose is converted into sugar, which, after fermentation by yeast fungi, forms ethyl alcohol.

By using modern methods of alcohol production from 7 to 8 gallons of alcohol can be obtained from 220 lbs. of dry wood.

The top fermentation yeast* used in alcohol production causes intensive fermentation, because the yeast strains are selected for their vigour and the ability to produce large quantities of alcohol.

Rice wine *saké* in Japan is produced by the mould fungus *Aspergillus oryzae* converting rice starch into sugar. Fermentation is caused by a mixture of common yeast and this fungus.

BREWING

Fermented beverages, not unlike beer, originated soon after man learned to till the land and cultivate cereal crops.

An Egyptian papyrus containing a message from father to son in which the latter is reproached for leading a dissipated life and drinking too much *gag*—a kind of beer known in ancient Egypt—has been preserved.

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* Yeasts are divided into yeasts of top and bottom fermentation. One property of the former is its ability to cause more intensive fermentation as compared with the latter, which is settled more easily

In the first century B.C. *cytos,* a spiced barley beer was brewed in Alexandria. Beer was known in Iberia, Thrace, Illyria and Pannonia (the territory of present-day Albania and Yugoslavia).

From Spain in the first century A.D. the art of brewing spread to Gaul, where beer, known as *cormé*, became a favourite drink.

As soon as Teutonic tribes began tilling the land they started brewing beer from grain.

Beer was brewed in all monasteries in the Middle Ages, and somewhat later also in the cities.

Brewing was well known in old Russia; the Russians, probably, learned to make barley malt for beer from the ancient Scythians (Virgil mentions their proficiency in the art of brewing).

Can beer be brewed without yeast, i.e., without microbes? No, it cannot, irrespective of whether it is made in the home or brewery.

The alcohol content of beer is low, not more than 4-5 per cent. Brewing is a long and complicated process.

Beer is chiefly made from barley malt.

Special strains of brewer's yeasts causing fermentation are added to the wort after the malt has been sweetened. Fermentation in special conditions continues for 9-10 days.

Then the new beer is drawn into sealed barrels to continue fermentation, to ripen and become aerated. The aeration with carbon dioxide causes the frothing of beer.

KVASS

Kvass is produced chiefly by mixing rye meal (or bread) with barley malt and water. Sugar produced in this mixture is split by the joint action of lactic acid bacilli and yeasts, producing lactic, carbonic and acetic acids and a small quantity of alcohol.

Usually only yeast is added to the mixture, for there is always some lactic acid bacteria in the materials used. Sometimes the bacteria are added, and then lactic fermentation takes a more regular course.

Hence kvass, too, cannot be prepared without the assistance of micro-organisms.

PRODUCTION OF VINEGAR

The term "vinegar" literally means "sour wine" (Fr. *vin*—wine, *aigre*—sour). Vinegar has probably been known to mankind since the discovery of wine-making, because weak wine readily sours and turns into vinegar.

Wine and beer are soured by acetic bacteria, which widely occur in Nature. The bacteria get into must along with ripe grapes, and once there, sour the new wine. They can also penetrate into wine or beer from the air.

Of the many species and strains of acetic bacteria (Fig. 36) those best suited for a given kind of vinegar are used in production. Vinegar is produced by different methods and from different materials.

In souring wine and beer, the bacteria cause fermentation of alcohol. This explains why vinegar in Germany was produced from strong beer and in Italy, France, Spain, Greece, and other countries, from grape wine.

Vinegar can be produced from any material, which, fermented, will yield alcohol, as for instance, apples, pears, plums, peaches, grapes, oranges, all kinds of berries, and honey. But grape vinegar produced from wine according to the Orleans method has always been considered the best. This method consists in filling a third of a 45-gallon barrel with first-grade strong grape vinegar containing a large quantity of select acetic bacteria capable of producing good vinegar. Then from 2 to 3 gallons of wine is poured into the barrel; a few weeks later the same quantity is added, the process being re-

peated three times at intervals of one week. A week after the last addition the vinegar is ready for use. From each barrel only 10-15 litres of vinegar are taken and an equal

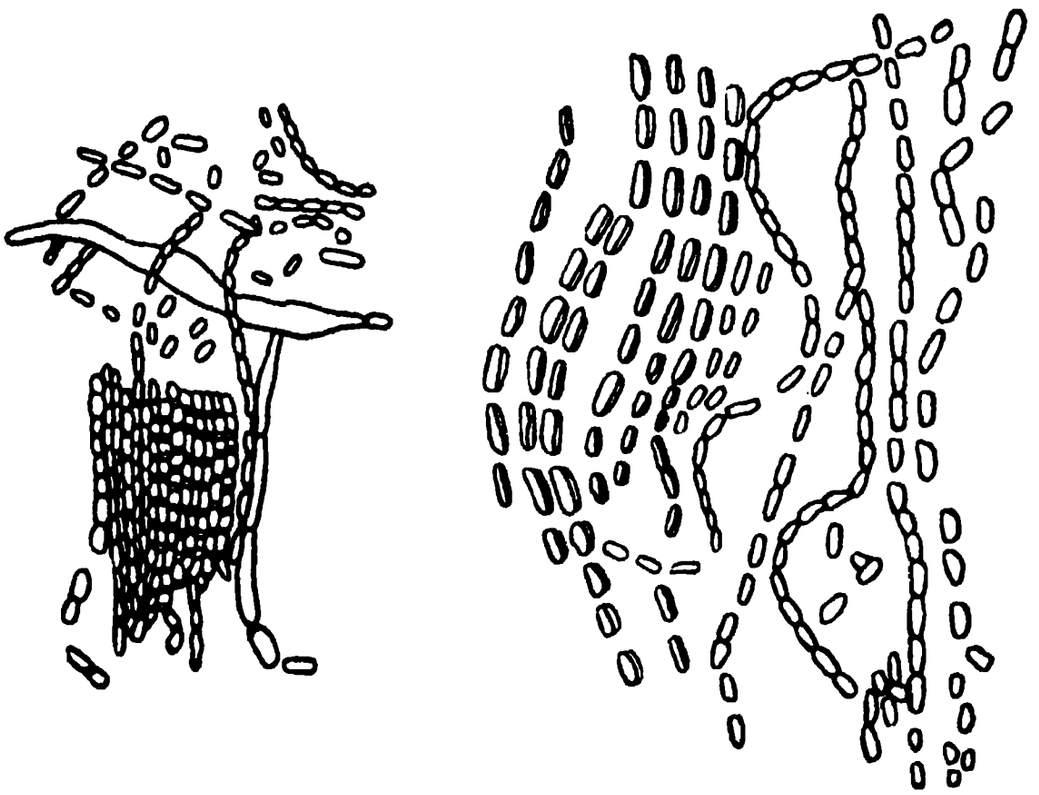

Fig. 36 Acetic bacteria

quantity of wine is added. Thus, week by week continues the slow but uninterrupted process of vinegar production.

At present vinegar is mainly produced from diluted spirits of wine by a method known as German, or the "quick generator process." This, too, is a continuous process lasting for dozens of years.

To accelerate the process of vinegar production by this method, large barrels or vats are filled with intertwined beechwood shavings which have previously been steeped in vinegar and become inoculated with acetic bacteria.

When vinegar is produced by the "quick" method, the alcoholic mixture contains 3 per cent of alcohol and 6 per cent of vinegar, and a certain amount of nutritive matter for the bacteria. Then the wort or gyle is poured into

the vats over the beechwood shavings. While it trickles through the packing material to the bottom, the alcohol is almost completely acetified by the bacterial action.

About two-thirds of the ready product goes for the preparation of a new mixture, and the remaining acetic acid is diluted with water, so that its content in table vinegar is 4.5 per cent.

As is known, vinegar is used in cooking and also for preservation of various vegetables, fruits, fish and other products to prevent their being spoilt by microbes.

BACTERIA IN MILK AND DAIRY PRODUCE

The very early cattle-breeders knew how to prepare dairy produce. Many of the tribes subsisted chiefly on sour milk and cheese.

Herodotus, the Greek historian who lived in the 5th century B.C., says that the Scythians prepared koumiss, i.e., soured and fermented mare's milk.

The first dairy produce man knew was probably naturally soured milk which had been exposed to air for some time. Later man learned to prepare other milk foods.

What causes the souring of milk?

Milk is soured by the action of the *Bacilli acidi lactici* which split sugar contained in fresh milk and produce lactic acid. The acid sours the milk, in which appear curdles—a sediment consisting of a protein known as casein.

All who are familiar with the life and work of Ilya Mechnikov know that he had devoted the last years of his life to the problem of combating old age, to the problem of longevity. He considered that the average span of human life should be about 150 years.

In studying this problem Mechnikov discovered that putrefactive bacteria inhabiting human intestines are

harmful to man, because they poison his organism with the products of their metabolism, i.e., their excretion. Since the bacterial flora of the intestines live in an alkaline medium, the best thing would be to have an acid medium in the intestines, unfavourable to bacterial growth. To this end he advised regular use of sour milk, the introduction into the intestines of lactic acid bacteria. Of various types of curdled milk he considered milk soured by the *Bacillus bulgaricus* to be the most effective.

Mechnikov chose the Bulgarian sour milk yogurt, because statistics showed that the percentage of old men of and over one hundred years of age was the greatest in that country. He attributed this longevity to extensive use of yogurt.

Mechnikov regularly ate this milk, otherwise known as lactobacillin.* But later research has proved that the human intestines do not provide the best environment for the *Bacillus bulgaricus* which does not create an acid medium there.

Not long ago another bacterium was discovered in the excretions of infants and calves; this bacterium producing a very high percentage of lactic acid in milk was, consequently, called acidophilous, i.e., acid-loving. The properties of this bacterium resemble those of the *Bacillus bulgaricus*, but, unlike the latter, it thrives in the human intestines. Lactobacillin, therefore, was superseded by acidophilin, soured by the acidophilous bacillus.

The Georgians and Armenians use sour milk called *matzoon*; this and other kinds of sour milk used in the south differ from the northern kinds in that they are prepared from boiled milk. The starter consisting of certain species of *Bacilli acidi lactici* and yeasts is added to hot milk (104° to 113°F.), which quickly turns sour. The *matzoon* ready for use is kept in the cold.

* Lactobacillin is usually prepared on "starters" consisting of *Bacillus bulgaricus* and *Streptococcus acidi lactici.*

Today there are special laboratories preparing starters containing mixed cultures of lactic bacteria. The microbes mentioned earlier make a better type of sour milk when used in symbiosis than separately; this type

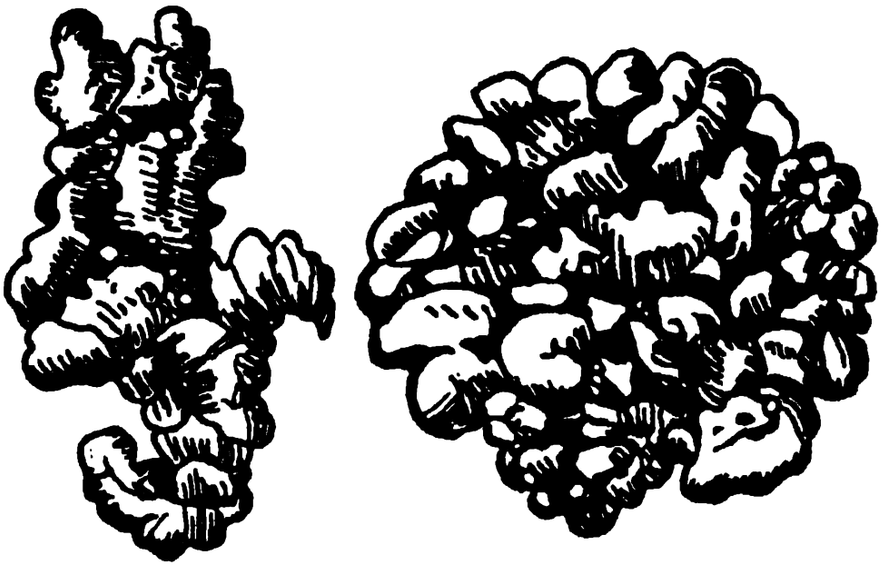

Fig. 37 Kefir grains (magnified)

of sour milk has a higher percentage of lactic acid and a much better flavour.

The souring of cream, too, is due to the action of lactic bacteria.

Kefir has been known for ages. It is of Caucasian origin, where starters containing kefir grains have been handled down from father to son for centuries. These grains, not unlike minute heads of cauliflower, are used for kefir production at dairy plants (Fig. 37).

Kefir grains consist of *Streptococci acidi lactici, Lactobacilli casei* and kefir yeasts producing alcohol and carbon dioxide, which account for good kefir's tart taste.

Home-made kefir in the Caucasian mountains ferments in well-tied up goatskins; at dairy plants kefir ferments in sealed bottles—that is why carbon dioxide does not escape and kefir becomes effervescent. Dried kefir grains keep well; when necessary, they can be wetted and used as starters.

Koumiss, mare's milk soured by a special starter,

katyk, has a peculiar taste. The starter contains lacto-bacteria of the acid-forming type, like the *Bacillus bulgaricus,* and yeasts of the *Torula* type. The bacteria produce lactic acid and the yeasts, alcohol. Koumiss also contains carbon dioxide, to which it owes its effervescence. The presence of alcohol accounts for the piquant taste of koumiss.

CHEESE

It would be natural to suppose that man learned to make cheese at the same time he began using for food curdled milk. The most important step in the production of cheese must have been the pressing of the sediment in milk to obtain curds. In order to keep the curds fresh, they were salted, and then pressed. In this way appeared the oldest and simplest type of cheese known as Bessarabian cheese or *brinza.*

This process, too, involves the action of micro-organisms. The lactic acid bacteria ferment lactose; lactic acid separates calcium from casein, which is converted into caseinic acid, insoluble in water and settling as sediment. The milk curdles and curds are obtained.

In some Oriental countries and in Africa cheese is to this day prepared in this primitive way, and then salted and dried to keep. Before eating, this cheese, hard as stone, is softened in tepid water for about two days.

It may have happened that cheese was not sufficiently well dried and bacteria and mould fungi multiplying in it changed its flavour, smell, hardness and other qualities, and thus spoiled the cheese. But sometimes the properties of cheese were altered for the better, it acquired a pleasant taste and aroma. This, probably, was the way ripened cheese was first produced.

How is cheese made today and what goes to make good cheese?

First and foremost, the milk for cheese must be

free of bacteria.* This is achieved by seeing to the cleanliness of the udder, the milkers' hands and the vessels in which milk is carried to the dairy plant.

Cheese contains the microbes which were present in the milk, and these influence its properties. That is why only bacterially-ripe milk, i.e., with lactic bacteria predominating over all other species, is used for making cheese. But besides lactic bacteria, milk naturally contains other micro-organisms making it more or less impure. All the same, lactic bacteria must constitute not less than 50 per cent of the whole bacterial population.

Rennet** added to bacterially-ripe milk together with lactic bacteria, converts it into a curd.

If cheese is made from bacterially-unripe raw milk, i.e., milk with an insufficient percentage of lactic acid bacteria, or pasteurized milk, bacterial starters are introduced in addition to rennet; the bacterial starter contains certain species of lactic acid bacteria—*Streptococcus acidi lactici* and bacilli, whose agency produces a pleasant flavour in cheese.

Rennet and the bacteria cause the milk to curdle at the first slight heating (up to 86°F.). During further processing, the lactic bacteria multiply in the curd at a very rapid rate.

The temperature of the second heating depends on the type of cheese prepared. The Holland and Latvian cheeses are heated to 104°F., a temperature at which microbes can still multiply in the curd. The Soviet and Swiss cheeses are heated to 131°-138°F.; at this temperature the *Streptococcus acidi lactici* is destroyed and the activity of other lactic bacilli is inhibited, while

* Bacteria-free milk contains minimum bacteria (such as *B. coli*, butyric acid bacillus, etc.) which get into milk from dung, the milkers' hands, the udder, etc. The presence of certain species of lactic acid bacteria is not regarded as impurity.

** Rennet is used in cheese-making in the form of dried and pulverized fourth stomach of calves.

that of the *Bacterium casei*, a thermophilic bacterium, is intensified.

After the second heating the curd is poured into moulds and compressed; the temperature at that stage is relatively high. Throughout the above processes, in spite of partial destruction of some microbes, their total number continues to increase. Thus while at the initial stage of cheese production the milk contains a few millions bacteria per one ml, at the end of the second heating the number of bacteria per one gramme of curd increases to hundreds of millions and after pressing reaches 1,000,000,000 per one gramme of cheese.

Along with beneficial lactic bacteria, harmful microbes, too, develop in unripe cheese, especially the *B coli*. Salting inhibits the development of harmful microbes and improves the flavour and hardness of cheese.

During the subsequent stage the ripening of cheese proceeds under the action of ferments produced by lactic bacteria. In some cases ferments of certain mould fungi also take part in the ripening.

The ripening of the Soviet and Swiss cheeses is brought about by the *Streptococcus acidi lactici* and the thermophilic *Bacterium casei*. The bacterial starters for these cheeses must contain these strains. The microbes must of necessity possess thermophilic properties, otherwise they would be destroyed by the second heating at 138°F.

In ripening cheeses of the Swiss type there develop bacteria producing propionic fermentation and liberating propionic and acetic acids which impart to ripe cheese its peculiar piquancy. Carbon dioxide escaping in bubbles of various size leaves holes in cheese which form the familiar pattern of eyes. Aromatic lactic bacteria, too, take part in producing this pattern.

Some species of yeast and mould fungi used in the production of soft cheeses lend to them a peculiar flavour and aroma. Thus we see that microbes play a leading role in cheese production.

K. Timiryazev, a well-known Russian naturalist, once said: "A slice of well-baked bread is one of the greatest inventions of the human mind." And indeed, many thousands of years had to pass before man learned to make good bread.

Humanity started growing cereal crops soon after the first settled habitations had appeared on earth, when hunting had ceased to be the only means of sustenance and the first steps were being made in breeding domesticated animals and tilling the land. Some scientists think this was about 15,000 years ago.

Bread as we know it today was not invented at once. First men ate raw grains, then began grinding them; later they toasted grains, crushed them and made porridge. Finally unleavened cakes were baked from ground grain. As there were no ovens, the cakes were baked on hot stones or between two flat stones or clay slabs. The earliest type of oven was a hole in the ground lined with stones and covered with a slab. The ovens of the ancient Hebrews and the neighbouring peoples were round holes of baked clay with sides a little above the ground, covered with bell-shaped lids, also made of baked clay.

Ovens of this kind are still to be found in the Middle East. Round ovens of baked clay but having no bell-shaped lid are to this day used in Georgia to bake *chureks*—a kind of flat cake.

But the quality of bread is determined by the properties of the dough rather than by the oven used. The ancient peoples who, thousands of years ago, inhabited Egypt, Greece and Palestine, noticed that if some fermented grape juice or a piece of old dough were added to flour, fermentation ensued, which made the dough porous and increased its volume.

Today fermentation in dough is known to be the result of microbic activity. But although in ancient times people

knew nothing of the existence of micro-organisms they utilized microbic activity for obtaining good bread.

What role do micro-organisms play in bread production?

In making wheat bread, some baker's yeast is dissolved in water and added to the dough. The yeast must be fresh, because fresh yeast fungi quickly multiply and produce active enzymes causing fermentation. The yeast ferments decompose the sugar contained in the flour and produced from the starch into alcohol and gaseous carbon dioxide which raises the dough and makes it porous.

During the fermentation process, lactic bacteria develop in the dough, which also decompose sugar, producing lactic acid, an important element in dough ripening.

The lactic bacteria and the acid they produce create favourable conditions for the yeast fungi, at the same time oppressing harmful microbes which might impair the flavour and aroma of good wheat bread.

Fermentative micro-organisms are active in the production of rye bread as well, but the microbes themselves and their activity are different. When dough is made from rye flour and water without yeast, fermentation is caused by the *Bacterium levans*, producing lactic and acetic acids, carbon dioxide and small quantities of hydrogen and nitrogen.

More flour and water are added to this dough, the process being known as the "rejuvenation" or "freshening" of dough. After "freshening" the *Bacteria levans* disappear giving place to lactic bacteria and yeast fungi which cause alcoholic fermentation with the escape of carbon dioxide. During fermentation the lactic bacteria produce lactic acid and a small quantity of acetic acid. As a result of the combined fermentation by the yeast and lactic bacteria, the dough becomes porous and undergoes some other ripening processes. This ripened dough serves as leaven for making rye bread.

The modern practice is to prepare rye dough with liquid yeast combined with leaven.

A few years ago a new method of quick rye dough preparation by means of a milk leaven and liquid yeast was worked out at the U.S.S.R. Bakery Institute.*

We see now that the bread we eat cannot be made without microbes—yeasts and certain species of bacteria.

Yeasts are produced at yeast plants. The baker's yeast consists of special strains of alcohol yeast fungi. The production of yeasts means their artificial breeding in immense quantities, sufficient to meet the needs of bread plants and bakeries of a district or town.

Yeast plants do not need expensive raw materials. The staple food for yeast fungi is a weak (1.5-2 per cent) solution of sugar obtained from molasses, a waste product of sugar refineries, to which are added nitrogen in the form of ammonia salts, and phosphorus.

At a medium-sized yeast plant a few pounds of living yeast are daily introduced into the fermentation vats, and the next day there develop several tons of young yeasts. The yeast fungi multiply at such a rate that in twenty-four hours their weight increases a thousandfold.

So we have to thank yeasts and lactic acid bacteria for the good bread we eat.

SAUERKRAUT AND PICKLED CUCUMBERS

Many people like sauerkraut and pickled cucumbers which remain juicy and crunch pleasantly as you bite them. But few know what makes cabbage and especially cucumbers, which are so apt to spoil, preserve their flavour.

The small quantity of salt added to the vegetable to be preserved (30 grammes salt per kilogramme cabbage) is

* L. Auerman, *The Technology of Bread-Making*, 1948.

meant not so much to destroy spoilage bacteria as to draw out the juices containing sugar from the cabbage or cucumbers. The sugar of cucumber juice is decomposed by *Bacteria cucumeris fermentati* and of cabbage juice by *Bacteria brassicae fermentatae*, two species of lactobacteria, producing lactic acid, carbon dioxide, a small quantity of alcohol and acetic acid, which give pickled vegetables a pleasant flavour and aroma. At the beginning of the pickling process some other bacteria develop in the brine, including spoilage organisms, but as the lactic acid content increases these are oppressed and the vegetables are preserved.

There are instances, however, when harmful fungi such as the *Oidium lactis* capable of decomposing lactic acid, develop in the brine. As the lactic acid content decreases, the conditions become favourable for spoilage bacteria, and the vegetables deteriorate. That is why it is advisable to inoculate the brine with a pure culture of lactic bacteria and in this way ensure the necessary lactic acid content, preventing harmful microbes from developing.

So we see that pickling would not be possible but for the existence of certain lactic bacteria.

SILAGE

The process of curing or preserving green fodder for domestic animals not only by means of drying, but by ensilaging, dates back to ancient Egypt or perhaps to an earlier date.

The crop to be ensilaged is stored in deep pits, trenches, wooden or stone towers. Silage may be prepared from maize, sunflower, Jerusalem artichoke, beet leaves, potato leaves, cabbage leaves, and various uncultivated plants.

When chopped green plant material is stored in a pit, trench or tower, various microbes, including a great

number of lactic bacteria, start to develop rapidly. The bacteria convert the sugar contained in the plants into lactic and acetic acids, the acidity of the fodder heightens and the spoilage bacteria which developed at the beginning of the process quickly die off. With further intensification of acidity, some species of lactic bacteria also perish.

With the ensilage process proceeding normally (in the absence of air) the product obtained possesses a very high degree of acidity which prevents it from spoiling.

Sometimes by adding a small quantity of common salt to ensilage the plants are made to exude more readily their sap containing sugar, which enhances the proliferation of the lactic acid bacteria. Well prepared fodder, especially if it is salted, possesses better nutritive qualities and is more pleasant to the taste than hay, which is deprived by drying of a large percentage of vitamins present in fresh forage.

Chapter VI

FOODSTUFFS AND FODDER SPOILT BY MICRO-ORGANISMS

BREAD

In summer wheat bread will sometimes develop an unpleasant odour of rottenness. This is apt to happen in southern localities. The inner part of bread becomes soft and sticky, there appear hollows in it and yellowish-brown or pink spots. The longer the bread is kept, especially in a warm surrounding, the more pronounced becomes its sliminess; the yellow colouring spreads all over it and then becomes brown. Anyone who breaks the loaf and sees the silvery threads inside will tell you that the bread is bad.

The poor quality of bread is not only due to bad flour or bad baking, but to the action of harmful bacteria which effect such alterations in the bread as to make it unfit for consumption.

The symptoms given above belong to the potato disease so named because the causative organism is the potato bacillus. *Bacillus subtilis*, also widespread in Nature, causes spoilage resembling the potato disease. Other spore-forming saprophytes, too, can cause bread deterioration.

How does bread become affected?

Bacteria of the potato bacillus and *B. subtilis* type occur in Nature in immense quantities. The potato bacillus is often present in flour, but its presence does not always cause the spoilage of bread. For the bacterium to cause harm, favourable temperature and humidity are necessary as well as moderate acidity. The potato bacillus

seldom develops in rye bread, for its high acidity is destructive to the microbe. On the other hand, the bacterium may be discovered in perfectly healthy wheat bread where it enters from the flour.

But why does baking not destroy the organisms? The potato bacillus forms exceptionally heat-resisting spores. During baking the vegetative forms of the microbe, capable of growing and reproducing, are destroyed, but their spores can easily withstand heating up to 212°F., the temperature within the loaf seldom exceeding the boiling point of water. When the baked bread cools, the spores germinate to produce new generations of the potato bacillus.

Besides this, there are other agents which spoil bread.

The *Oidium aurantiacum* and *Thamnidium aurantiacum* fungi produce in bread red or orange-red flakes; the inside of bread becomes red or marbled with a yellow-white pattern. Bread affected with these fungi is not harmful. Sometimes white spots or mealy patches appear in bread; after a time the spots and patches dry up and turn into a powdery substance resembling chalk. This is the "chalk disease" caused by a species of yeast fungus. "Chalky" bread does little harm but its commercial value is impaired.

Mould on bread is familiar to all. White or black mould is caused by the *mucor* fungi while the *Penicillium glaucum* produces a bluish-green mould. Other mould fungi attack bread less frequently. Moulding bread has an unpleasant musty odour, often turns bitter and cannot be used for food.

The "intoxicating bread" infested with the *Fusarium gramineaurum* fungus, as well as the ergot-affected flour and the grain kept under snow during the winter are described in Chapter III.

Micro-organisms are also responsible for the souring of flour and spontaneous heating of flour and grain.

SPONTANEOUS HEATING OF GRAIN, FLOUR, SILAGE AND HAY

The spontaneous heating of flour and grain may be due to various causes. It may be due to excessive humidity of the grain or flour. Too high humidity even of a part of stored grain activates micro-organisms and grain ferments, and is accompanied by generation of heat. If

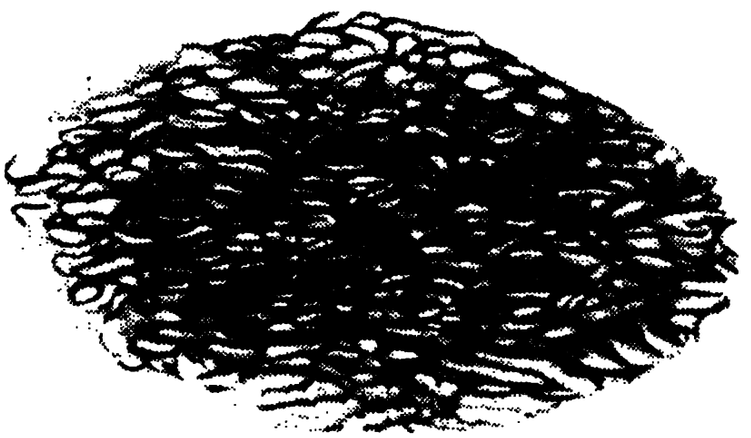

Fig. 38 A solid piece of charred grain (after E. Mishustin)

this process occurs in the lower layers of grain, the generated heat cannot escape and a hot centre is formed. As the temperature of the flour or grain gradually rises the number of thermophilic microbes increases, while that of other organisms diminishes. This process may bring the temperature up to 140° and even 176°F. The grain is turned into a charred, almost black solid mass, which can be broken only with a pick or crowbar (Fig. 38).

Numerous investigations show that thermophilic microbes play a very important role in spontaneous generation of heat. To prevent their development, grain must be dried well and treated with dichlorethane before storing.

If packed hay is not quite dry, it becomes heated deep inside the rick, and if no measures are taken, may even

catch fire. The spontaneous ignition of moist hay has also been traced to the activity of microbes, especially thermophilic ones.

The same is apt to happen to silage which has been packed loosely. If conditions are favourable thermophilic micro-organisms cause not only spontaneous ignition, but even explosions of such strength as to destroy ensilage towers.*

SPOILAGE ORGANISMS IN MILK AND DAIRY PRODUCE

Besides microbes which help to convert milk into various dairy products and cheese, there are many micro-organisms which spoil them.

If strict cleanliness is not observed in milking the milk smells of the byre and may contain bacteria inhabiting the digestive tract of man and animals, which get into it along with particles of manure.

Many micro-organisms, such as mould fungi and *B. coli*, lend to milk a turnip flavour. Sometimes the milk has a soapy taste, which is due to the presence of the soap bacillus.

Milk that has been kept long in the cellar often acquires a bitter taste, brought about by the spoilage bacteria which are active at fairly low temperatures. They decompose proteins and produce bitter-tasting peptones.

When thermophilic streptococci, the ropy milk bacterium or the cloacal bacterium, separately or together, get into milk, it becomes ropy. The ropiness of acidophiline has nothing in common with that property caused by spoilage bacteria.

Pigment-producing bacteria may render milk blue, red or yellow (see Chapter II).

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* E. Mishustin, *Thermophilic Microbes in Nature and Practice*, U.S.S.R. Academy of Science Publishing House, Moscow, 1950.

Milk becomes red under the influence of the *Bacterium prodigiosum* we are already familiar with. The development of the *Bacterium cyanogenes* in milk gives it a blue tint. These bacteria are aerobic and develop in the upper layers of milk, dyeing it with the pigment they produce.

Pathogenic microbes can also get into milk from a sick cow, ewe or any other animal. The milk of a tuberculous cow contains tubercular bacilli, goats and ewes affected with brucellosis produce milk contaminated with brucellae (see Chapter X).

Dairy products, too, are spoiled by microbes; there are various forms of butter and cheese destruction. The most common form in cheese is distension. Fissures appearing in a hard cheese at an early stage of production spread to the surface and cause the rind to crack. Then bacteria and mould fungi get into the cheese and cause its decay and moulding. Or coliform bacteria may get into the milk as a result of slovenly milking, and produce gases (mostly carbon dioxide) in the cheese, whose presence makes the cheese distend.

Butter is commonly affected with rancidity which is the result of an oxidation process splitting fats into glycerine and fat acids, which are further decomposed by micro-organisms. The butyric acid produced during decomposition imparts to butter a bitter flavour.

Butter may have a nasty smell, or a flavour of cabbage or turnip, if coliform bacteria get into the cream from which the butter is churned and if the temperature is favourable for their development. Fluorescent bacteria impart to butter the flavour of vegetable or mineral oil. Coliform bacteria and certain yeasts may produce large quantities of gas and cause the distension of butter.

If butter is kept in an atmosphere of high humidity it begins to mould. Caskets, crates and oil-paper contaminated with mould fungi infect butter. These moulds most often attack sweet butter.

PUTREFACTIVE MICROBES

Putrefactive bacteria, such as the *Proteus vulgaris*, *B. coli*, and others, cannot attack the tissues of a living organism, for living cells are able to defend themselves against these microbes. But as soon as death occurs the organic tissues lose their defensive qualities.

Investigation has established that the muscles of a healthy live animal are free from micro-organisms, i.e., are sterile. But if the dressing of the slaughtered animal is conducted in insanitary conditions and if the internal organs, and particularly the intestines, are not removed immediately, immense numbers of bacteria multiply in the meat within a few hours. How does this happen? The intestines of any healthy animal are inhabited by countless numbers of bacteria, in fact thousands of millions per gramme of faeces. In a dead animal the walls of the intestines no longer resist the spread of the bacteria into other organs and tissues. The surface of a carcass becomes inoculated with microbes if the skinning is done carelessly for the animal's wool contains at least 500,000,000 micro-organisms per gramme.

Putrefactive organisms usually develop in the upper layers of the muscles (meat) and penetrate inside slowly. But, on the surface of the meat, while it is moist, conditions are most favourable for bacterial development.

With the temperature of the atmosphere of about 68°F. the bacteria decompose meat at a fairly rapid rate. The meat is covered with slime, becomes soft and acquires an offensive smell, because proteins, the main components of meat, decompose with the release of hydrogen sulphide, ammonia and other malodorous gases.

Most putrefactive bacteria are aerobic, that is they can only live in oxygen, but sometimes the meat begins to rot at the bones. This process occurring inside the tissues without any oxygen is the result of the activity of anaerobic organisms.

A good housewife can tell at a glance whether fish is fresh. Bad fish has opaque, slightly hollowed eyes, its gills are a dull grey and slimy; the fish is soft, yields easily to a touch, floats on the surface of water, and if the process is far gone has a characteristic odour of decay.

Fish affords better conditions for the development of microbes than meat. Fish, as a rule, is not gutted, unless it is processed at a fishery. That means that the inexhaustible source of micro-organisms—the intestine—is always present in the fish. The scales, too, are almost always covered with slime, the breeding-ground for microbes. The third source of microbes in fish are the gills filled with blood, which is a favourable medium for bacterial growth. These micro-organisms find their way into the flesh of fish much easier than into that of animals, as fish usually get skin injuries, especially if caught with hook tackle.

In the latter case not only putrefactive bacteria, but also harmful microbes, such as the botulinum organism, can get into the flesh. This micro-organism produces a toxin causing a strong poisoning which is often fatal.

Putrefactive micro-organisms which along with other microbes are always present in water can develop in fish at lower temperatures than in meat.

That is why fish gets spoilt more readily than meat. Besides there are infectious fish diseases, and diseased fish spoils more quickly than healthy fish. Fish affected with furunculosis and carps affected with "the red disease" are particularly apt to deteriorate.

A fish product that easily goes bad is fresh caviar—unfertilized spawn. While in the fish's body the spawn is sterile, the moment it is taken out it is attacked by microbes from the hands of the operators, from the vessels, the water, etc. If caviar were not preserved it would decompose within a very short time, for it is a highly nutritive substance and affords an excellent medium for the development of putrefactive microbes.

Eggs are well protected by their lime shell and the integument that is beneath it, but they, too, do not keep fresh long.

It is easy to see whether an egg is fresh by holding it over an ovoscope or against the sun. If no dark spots are visible the eggs are fresh. Another sign is the smell—a bad egg has the characteristic odour of hydrogen sulphide, usually known as "the smell of rotten eggs." How does this smell originate? The putrefactive bacteria which penetrate into the egg through the pores in the shell decompose the white, producing, among other substances, hydrogen sulphide.

Apart from putrefactive organisms, the spores of mould fungi may also penetrate through the shell into the egg where they germinate and form black spots. A mouldy egg has a musty odour.

Mould and yeast fungi spoil also fruits and berries.

* * *

The second half of the 19th century witnessed a rapid development of the sugar industry in Russia, but production was hampered by waste. During the boiling of sugar, the syrup in the vats became covered with slime, gradually spreading and filling the vats, until syrup was reduced to a viscous slimy mass. Many scientists tried in vain to solve the problem of this spoilage which caused such immense losses to the industry.

L. Tsenkovsky, one of the first Russian microbiologists, turned his attention to the question. He analyzed the slime and discovered that it consisted of a species of cocci encased in a gelatinous capsule. The bacteria decomposed sugar and, multiplying, filled the entire vat (Fig. 39).

When the cause was discovered it was not long before methods of protecting sugar syrup against the microorganisms were found.

Microbes may also affect sweets. Chocolate-iced candy, for instance, attracts yeast fungi which decompose sugar and release gases, especially carbon dioxide. The sweets distend and crack. The distension is most often observed in iced sweets, because the coat of icing prevents the escape of the gases through the pores. Sometimes mould fungi develop also on sweets and, like yeast fungi, easily adapt themselves to high concentrations of sugar.

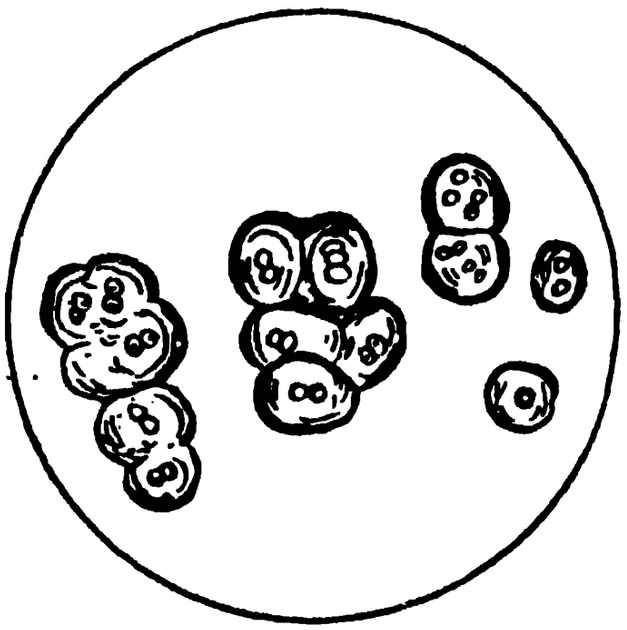

Fig. 39 The *Leuconostoc mesenterioides* cocci decomposing sugar syrup

One of the causes of yeast and mould fungi developing in sweets are insanitary conditions at the factory: dirty raw materials, vats, etc. Cleanliness is especially important in shops where sweets are cold-processed, because boiling destroys all vegetative forms of microbes.

Dampness in the storage premises is also harmful to sweets. Packing materials are of considerable importance: if sweets are packed in oil-paper which affords no outlet to moisture accumulating in sweets, the air in the package becomes moist and stagnant, which is conducive to the development of mould fungi.

DISEASES OF WINE

Pasteur was the first to discover that there are a number of diseases of grape wines caused by various microorganisms. Some of the diseases are the result of the activity of aerobic bacteria, as for instance, the souring

of wine and its conversion into vinegar by the action of the acetic bacteria.

Another well-known disease of wine is caused by the mycoderm organism—a mould fungus which produces a characteristic film on the wine and destroys alcohol, the extractive substances of wine, and sometimes also organic acids.

Propionic acid fermentation occurring deep in the vat or barrel without access of air is a serious wine disease. The symptom of this disease is the formation of silky turbidity in wine and, at an advanced stage, a peculiar "mousy" odour. The anaerobic rod effecting fermentation is encountered in both red and white wines.

Chapter VII

PROTECTION OF FOODSTUFFS FROM SPOILAGE MICRO-ORGANISMS

It has been mentioned earlier that drying creates unfavourable conditions for microbes, and though it does not always destroy them, drying reduces them to a state of inactivity in which they can do no harm. Naturally, neither our forefathers nor modern nomadic tribes knew anything about the existence of microbes, to say nothing of their properties. And yet preservation of meat, fish and fruit by drying has been practised since ancient times.

Today cured fish and dry fruit are used for food, and stock-breeders in the highlands preserve meat by drying.

Salting is a good method of preserving many perishable products from being spoilt by microbes. But there are microbes which can live in brines of high salt concentration, and against these salt is ineffective. To protect salted fish and meat against such microbes, they are kept in refrigerators; this will be dealt with at greater length elsewhere.

And there are halophilic (salt-loving) microbes which survive in brines of a very high concentration (up to 35 per cent of salt) and can even live on salt crystals.

Sometimes gutted and well-salted fish—zander, carp, bream, Caspian roach—are not transported in a brine but are simply sprinkled with salt. If you enter the storehouse where the fish are kept you will scent a very unpleas-

ant odour. The fish from which this odour issues have a reddish film inside, which sometimes covers the fish also outside. The film consists of specific bacteria which can live only in highly concentrated salt solutions. They may develop on salt, and sometimes salt mounds are pink, or even red. The bright red of colonies of these bacteria (*Serracia salinaria*) has earned them the name of fuchsine—by analogy with the aniline dye of that name used in microbiology.

Fish infested with fuchsine possess an unpleasant smell and taste and may cause diarrhoea in man.

Fish, slightly infested with fuchsine, should be washed well, since water removes most of the bacteria and kills the rest which cannot live in weak salt solutions. The fish must be well cooked before it is fit for consumption.

The fuchsine organisms can develop on other salted substances, as for instance, salted guts ("red guts") and on salted hides and skins, etc.

But if fish is preserved in brine the bacteria will not develop, for they require atmospheric oxygen. Nor do they develop in refrigerators, because low temperatures destroy them.

Although salted fish and other foodstuffs are liable to get spoilt, salting of perishable foods is a widely practised method of preservation.

Salted fish, ham or fowl taste better and are preserved better when smoked. Smoking has also been known for centuries. This method inhibits the development of putrefactive organisms, for the smoked product becomes partly desiccated. Formalin, resins and other substances present in smoke impregnate the smoked product and prevent the growth of the organisms.

High concentrations of sugar also prevent micro-organisms from developing, and that is why jams and candied fruit keep well.

PICKLING

Pickling is a popular method of preserving foodstuffs, for most putrefactive bacteria cannot develop in an acid medium.

The addition of vinegar to the brine preserves fish, mushrooms, cucumbers, grapes, plums and other pickled products from becoming spoilt.

But the brine should be carefully prepared as various microbes, including vinegar bacilli, certain mould fungi, and other micro-organisms which develop in mildly acetic media, may get into the brine and spoil the product.

CANNING

The method of preserving fish, meat and vegetables by heating was discovered about 150 years ago.

Canning—the preservation of perishable products such as fish, meat, game and vegetables for an indefinite time —is based on sterilization, i.e., on destroying putrefactive and other injurious microbes present in the can by high temperature treatment.

The foodstuff to be canned, as fish, for instance, is washed, cut into slices, seasoned with salt, pepper, onions, etc., and put into cans or glass jars which are then sealed so as to be perfectly air-tight. Then the cans or jars are placed in an autoclave and under pressure subjected to heating at a temperature between 212° and 248°F. Sterilizing destroys the putrefactive bacteria and keeps the food fresh and wholesome.

Sterilized canned foods can keep for a considerable time in a dry store-room.

Sometimes, however, the spores of the most heat-resistant bacteria remain in a can after heat-sterilizing and this residual microflora may reproduce and spoil the food.

Such residual organisms produce gases which often distend can ends; this is not very dangerous, because a "flipper" can is easily detected. But sometimes there are no apparent signs of any defect or of the presence of bacteria in the food which may cause poisoning: the can and the food it contains look perfectly normal and yet the *Clostridium botulinum* may have entered the can together with the food (see Chapters VI and X). The botulinum organism produces an exotoxin which does not change the appearance of food in any way, but the minutest quantity of which causes death.

That is why sanitation and strict microbiological control in the canning industry are of such paramount importance.

The well-developed canning industry in the U.S.S.R. is eqipped with up-to-date machinery and strict control is exercised over the processing of food at its enterprises.

COLD STORAGE

That meat, milk and other foodstuffs keep well if stored in a cold cellar in summer was known long ago, but people thought that warmth was responsible for their decay. They did not as much as suspect that warmth was conducive to the development of putrefactive bacteria, the true cause of putrefaction, although as far back as the 18th century Plenciz (see Chapter I) spoke of microorganisms as the agents of rotting.

It was only 85 years ago, in 1870, that Charles Tellier, a French scientist, published his discovery that meat could be preserved fresh in the cold produced by special machines. His experiments were applied in industry in 1874 when the first cooling machine was constructed. Soon packing-plants spread in many countries.

Modern packing-plants are many-storied buildings containing a number of rooms whose walls are lined with a thick layer of a porous insulating material preventing heat from penetrating from the outside and cold from leaking into the atmosphere.

The rooms are cooled by pipes resembling central heating in homes, the pipes containing cold ammonia or brine instead of hot water. In many packing-plants the cold air is forced into the rooms through special tubes (air coolers).

The temperature in the rooms is controlled so that different food products are stored at different temperatures.

Chilled products, such as meat, milk, eggs, fruit and vegetables which are kept for a short time (the so-called short-carry) are preserved at 32°F. Other rooms are held at —0.4°F. Meat and fish (long-carry) are frozen in special refrigerator-rooms at —7°F. until they are hard as stone. In this state they can be kept in a packing-plant for years, and although they dry up in the course of time, they can be cooked and fried, just like fresh meat and fish. The flavour and nutritive value of such products are largely preserved.

The technique of cold storage has been so improved that there is always a steady supply of well-preserved foodstuffs, and shops have on sale fresh-frozen fruit, peas, berries, etc., in winter and fresh meat and fish in summer.

Foodstuffs preserved in cold storage are not entirely free from microbes. Quite a number of micro-organisms, especially mould fungi, are well adapted to low temperatures and continue to live and reproduce in packing-plants. The organisms are brought into refrigerators mostly on the fresh products. Thus, for instance microbes brought to the packing-plant on the surface of meat are not killed there, but their development is somewhat

inhibited. It sometimes happens that after freezing the carcass becomes covered with mould, but owing to the low temperature the mould spreads slowly and does not penetrate deep into the meat.

Although no mould fungi can be seen on animal fats preserved at 3°F. to —0.4°F., they eventually acquire a pungent musty odour. Therefore, the storage temperature for butter and other animal fats has to be still lower.*

* A. Voitkevich, *The Microbiology of Milk and Dairy Produce*, Moscow, 1948, pp. 224-33.

Chapter VIII

MICROBES IN CHEMICAL INDUSTRY

BUTYRIC ACID

Butyric acid which appears in spoilt butter accounts for its rancid flavour and odour. Butyric acid is formed in the process of decomposition and it appears in pickled products, during the retting of fibrous plants, etc. It is the product of the activity of different microbes, including the butyric acid bacteria which cause the fermentation of certain carbohydrates. Spontaneous production of butyric acid is undesirable, but some of its alcohol compounds (ethers) have the odour of rennet apple, pine-apple, pear, etc., and are widely used in the perfumery and confectionery industries for production of sweets, juices and soap.

The production of butyric acid ethers, secured from vegetable starch (potato, cereals, etc.) and the residue of the sugar and starch industries, such as molasses, is now an important industry. Starch is converted into sugar by the application of a weak solution of sulphuric acid and the resulting substrate is then inoculated with a culture of butyric acid bacteria causing fermentation, which produces calcium salt of butyric acid. Then butyric acid is obtained by chemical methods, but since pure butyric acid finds no application in industry, it is etherified and the ethers obtained are used in the perfumery and confectionery industries.

Thus, through the activity of certain microbes we have toilet soap, various sweets and lemonade.

CITRIC ACID

Natural citric acid extracted from lemons and other citrus fruits is very expensive. However, the study of the properties of mould fungi has shown that such fungus species as the penicillium and aspergillus can produce citric, malic, oxalic, and other acids.

Today microbes are utilized for the industrial production of citric and other acids, because the microbiological method is much cheaper.

In the U.S.S.R. citric acid is produced at special plants where the rational microbiological methods are based on the researches of S. Kostychev, V. Butkevich and other scientists.

LACTIC ACID

We have already dealt with the role played by lactic acid in dairy and other practices. Besides the food industry, lactic acid is important for other branches of industry.

In the textile industry—in printing and dyeing—lactic acid is used as a mordant, along with tannin. In the tanning industry it serves as the agent for removing lime from leather. It is also extensively applied in medicine—in the production of drugs and, in pure form, as a cauterizer.

To meet the big demand for lactic acid, it is now obtained commercially by the simple method of bacterial fermentation of sugar.

Lactic acid can be produced in different ways. By the Shaposhnikov method industrial waste, such as molasses, sunflower and hemp oilcake, is used for raw materials. The fermentation is produced by pure cultures of special thermophilic lactic acid bacteria. The method is cheap and highly productive.

GLYCERINE

Formerly glycerine was produced from animal fats. But it was Pasteur who discovered that glycerine was a by-product of alcoholic fermentation. Today glycerine is produced by adding 2-3 per cent of sulphite to a liquid fermented by yeast fungi.

ACETONE AND BUTYL ALCOHOL

Acetone is widely used in the chemical industry and in the manufacture of explosives. It can be obtained both by chemical and microbiological methods. When the latter method is used the products of fermentation are aceton and butyl alcohol, which also has industrial applications. Butadiene, a substance required for the production of synthetic rubber, is prepared from butyl alcohol; butyl alcohol is used as a solvent in the production of nitrocellulose varnishes. This shows the economic importance of acetone-butyl fermentation.

A thorough study of the problem at the Shaposhnikov laboratory in the U.S.S.R. has revealed that acetone-butyl fermentation is caused by the anaerobic acetone-butyl bacteria.

The raw materials are flour (rye or maize) and potatoes.

Today acetone and butyl alcohol are manufactured at factories with bacterial agency.

MICROBES AND THE TEXTILE INDUSTRY

The home method of retting flax and hemp practised from ancient times is to lay the stalks on the ground under the open sky.

The fibrous plant spread on the ground is alternately acted upon by dew and rain, heat and light, and all the time it is exposed to the air. Various aerobic micro-organisms multiply on the stalks.

Dew-retting frees the bast bundles from cellulose and rind, but it is a lengthy process, so the Soviet scientist V. Omelyansky suggested a quicker method known as water-retting. Here flax or other fibrous plant is steeped in a stagnant pond, a slowly-running river or in special vats and is worked upon by anaerobic bacteria, the flax-retting bacilli.

In 1895 V. Fribes, a Russian research worker at the Vinogradsky laboratory, discovered the bacillus *Plectridium pectinovorum* which for a long time was considered the organism of flax retting. But it was proved later by the Soviet scientists G. Seliber and his co-workers that this bacterium occurs only at an early stage of retting, and that advanced stages are characterized by the appearance of great numbers of the cigar-shaped rod *Clostridium felsineum.*

What then is the role played by aerobes in dew-retting and anaerobes in water-retting?

Parts of the stalk in plants, particularly in flax, are cemented by a substance called pectin. During retting the bacilli cause pectin to ferment and thus destroy it; the parts of the stalk are no longer held together and when flax is scutched and combed the fibres are freed from shives and peel and become separated one from the other.

Further investigations in this field by Soviet scientists (G. Seliber and others) have made it possible to improve retting methods. One of the new methods consists in combining the aerobic retting by means of sprinkling with the anaerobic retting by submersion. By this method flax is subject to both aerobic and anaerobic bacterial action.

FINISHING TEXTILES

Fabrics are continually subjected to starch-removing processes during manufacture. This process is known as finishing or sizing.

A number of amylolytic bacteria are capable of dissolv-

ing starch. A. Imshenetsky and his co-workers recently offered a new preparation which they called biolase, containing the thermophilic ferment, amylase, capable of dissolving starch and converting it into dextrine, which in turn becomes progressively converted into maltose and glucose. The two types of sugar are then easily dissolved in water.

TANNING LEATHER

The leather used in shoe manufacture and in the production of many other goods has to be waterproof. This is achieved by tanning—a process which results in impregnating leather with insoluble substances. In addition, tanning lends to leather elasticity and preserves it from decay.

Fig. 40 Gall-nuts on an oak leaf

Gallic acid forms part of many complex chemical compounds. Its percentage is especially high in tannin. Substances containing tannin—tannides—are widespread in Nature. All fruits which possess an astringent flavour—tea, dry red wines (particularly, Kakhetian)—contain tannin. It is also found in the bark of some trees, such as willow, young aspen, oak, etc. Often globular excrescences can be observed on oak leaves; these are galls (or gall-nuts) formed from leaf tissue by certain flies. Galls are very rich in tannin and are widely used for making ink which must contain tannin (Fig. 40).

Tannin from galls and gallic acid from tannin can be obtained chemically, but the bacteriological method is simpler. It makes use of the ability of certain fungi, in particular the mould fungus black aspergillus, to decompose tissues with the production of gallic acid.

The bacteriological method of producing gallic acid directly from tannin-containing plant material consists in crushing gall-nuts, adding some water and inoculating the pulp with the spores of black aspergillus. A fortnight later pure gallic acid produced by the action of tannase, a ferment of the fungus, is extracted from the mass by the application of hot water or a mixture of alcohol and ether.

FERMENTATION OF CELLULOSE

Chemical analysis has shown that the walls of plant cells consist mainly of cellulose, one of the most resistant plant constituents (second only to lignin). Yet cellulose, too, can be decomposed.

Soviet scientists have established that cellulose is decomposed by special microbes which, accordingly, were named cellulose. This group includes cellulose-methane and other bacteria. Dozens of various fungus species, such as fusarium, chetomium, and some others, also contribute to cellulose decomposition.

The activity of cellulose micro-organisms in Nature can be observed wherever accumulations of vegetable remains are formed—in soil, silt, manure, sewage, compost heaps,* rotting sawdust and in the intestinal tract of herbivorous animals consuming great amounts of cellulose in plant food. Herbivorous animals do not produce ferments splitting cellulose, but their intestines

* Compost heaps or stacks are made to produce fertilizer. Garbage and waste piled up in heaps are decomposed by thermophilic micro-organisms, and many pathogenic microbes perish in the process.

are inhabited by microbes which perform this function and facilitate the assimilation of coarse food. Sometimes cellulose microbes are encountered in the intestinal system of carnivorous animals, and even of man.

Cellulose microbes are extremely active in soil where they decompose large quantities of vegetable matter.

The products of cellulose decomposition are the mother product for humus. When the process of cellulose decomposition takes place deep in the earth, hydrogen and methane are accumulated; the accumulations of these combustible gases are sometimes so great that they are used for industrial and everyday needs, like coal gas.

The organic (plant and animal) matter contained in sewage waters coming from human habitations and industrial enterprises is acted upon by various micro-organisms, including cellulose bacteria, and is finally reduced to simple mineral compounds.

It should be noted, however, that cellulose microbes are not always beneficent. Research has shown that these organisms destroy paper which consists mainly of cellulose. The organisms constitute a grave danger if they develop in libraries or archives where they may destroy valuable books and historical documents. While inspecting a big library in 1928 V. Bakhtin discovered coloured spots on pages affected with cellulose fungi; sometimes pages were stuck together, or had become brittle; there were cases of discoloration. Some species of microscopic fungi occur most often and seem to be the most dangerous enemies of books.

Cellulose micro-organisms develop most readily in badly heated, damp and insufficiently ventilated libraries and archives. That is why air-conditioning is practised in the Lenin and Saltykov-Shchedrin public libraries in Moscow and Leningrad, as well as in other important book repositories. There are also special laboratories for the protection of books and documents preserved in the public libraries.

Chapter IX

MICROBES HARMFUL TO FUEL, METAL AND WOOD

Besides foodstuffs microbes are also liable to spoil metal, wood and other building materials, as well as peat and coal.

Workers in the fuel industry know of spontaneous heating and even ignition of peat and coal.

Observations prove that spontaneous heating occurs mostly in briquetted peat—a well-dried porous substance piled together with free circulation of air. Spontaneous heating is caused by the activity of certain species of bacteria, fungi and actinomycetes, chiefly thermophilic, developing at temperatures between 122° and 167°F.

Within the pile of peat the temperature may reach 158°-176°F. If the process of spontaneous heating goes on for any length of time (from 3 to 8 months) the peat is reduced to a charred highly porous and brittle mass known as semicoke. The formation of semicoke may be accompanied by spontaneous ignition.

Coal, too, is subject to spontaneous heating and combustion.

E. Mishustin describes the following incident in his *Thermophilic Microbes in Nature and Practice:* Four ships carrying a cargo of coal were at sea; the coal was observed to heat. In order to cool it, ventilation was intensified in three of the colliers. The effect proved the reverse of what was intended. Whereas before only sparks were observed, those were now fanned into flame and three

ships were destroyed by fire. The holds in the fourth ship were sealed tight, and therefore the heating did not develop into combustion and the ship was unharmed.

All who have to deal with pipelines buried in the ground are familiar with corrosion, which is a frequent phenomenon. Corroded pipes begin to rust in places and have irregularly-shaped hollowings from 1.0 to 6 millimetres deep. Corrosion sometimes affects large sections of a pipeline and may cause serious leakage (of oil, for instance). When this happens in a water-supply system, microbes from the soil get in and pollute the water. In the case of a gas pipeline leakage may cause explosions and fires.

In order to avoid the harmful consequences of corrosion, pipes should be kept in good trim, and when necessary, changed. The over-all length of pipelines in all countries is well over 310,500 miles and vast sums are spent on the maintenance of the lines. In the 30's pipe corrosion in the U.S.A. caused annual losses of 100,000,000 dollars and in Germany from 1,000,000,000 to 2,000,000,000 gold marks.*

Certain bacteria inhabiting the soil in which pipes are laid play a considerable part in causing their corrosion. The bacteria start the process of oxidation on the metal surface of the pipes and the reduction of sulphuric salts in the soil. The resulting hydrogen sulphide combines with the metal of the pipes and produces ferric sulphide. All this destroys the walls of the pipes, which is proved by their diminished weight.

Today effective methods have been elaborated to combat corrosion, such as coating the pipes with bitumen with an electro-chemical (catode) layer.

Sometimes *Crenothrix polyspora*, a species of iron bac-

* L. Rubenchik, *Micro-Organisms as a Factor of Concrete and Metal Corrosion*, Ukr. Academy of Science Publishing House, Kiev, 1950.

teria, gets into a water-supply system. The organisms attach themselves with their filaments to the inner walls of the pipes and form minute plugs of iron oxide which may completely choke the service pipes.

Soviet scientists have established that bacterial activity may also prove harmful to brick and concrete constructions. Academician B. Isachenko has observed the weathering of brick walls of houses, bath-houses, conservatories and other buildings. On the crumbled walls he discovered layers of crystals of sulphates and nitrates. He thinks that the destructions were due to the activity of the sulphur and nitrifying bacteria found on the walls. Smoke escaping from furnaces and settling on damp walls contains hydrogen sulphide which is oxidized by sulphur bacteria with the release of sulphuric acid.* Nitro-bacteria produce nitric acid, and the two acids act on the material and plastering of the walls and destroy them.

Concrete is an extremely durable building material but concrete constructions are also subject to gradual weathering. Research shows that besides physical and chemical causes, micro-organisms, too, are responsible for these destructions. Nitro-bacteria and some fungi produce acids destructive to concrete and cement.

* * *

Rubber, both natural and synthetic, is a very important industrial product. But sometimes rubber is covered with white or red spots, it becomes brittle and perforations appear on its surface. This is caused by two species of actinomycete (ray fungi) and some species of mould fungi of the genera penicillium and aspergillus.

Persistent research has led to the discovery of one more bacterium whose development deprives rubber of up to 10 per cent of its initial weight.

* * *

* *Ibid.*

A very unpleasant phenomenon sometimes occurs in a wooden house: a nail driven into a wall goes right into it as if it were dough and can be extracted with the fingers out of the rotten wall.

The rotting of wood is caused by *Merulius lacrimans*, so named because it produces a tear-like slime in the wood. It is also called wood fungus.

If the presence of the fungus is not discovered in good time the house may rot and crumble. The fungus may also develop in the wooden parts of a brick house—wherever parts of its mycelium can penetrate.

The wood fungus is not the only organism harmful to wood. There is the "brown rot" caused by some microscopic fungi capable of decomposing celluiose. Wood affected by these fungi becomes so brittle that if rubbed between finger and thumb it crumbles to powder. One of these fungi, *Poria incrassata*, often attacks pine buildings causing enormous damage.

Chapter X

MICROBES CAUSING INFECTIOUS DISEASES IN MAN AND ANIMALS

Infectious diseases are communicable from sick person or animal to those that are sound.

A person ill of cholera can communicate the disease to healthy people by means of direct contact with them or through the things he had handled, if no preventive measures had been taken.

Infectious diseases are caused by pathogenic microbes. But a person into whose organism a pathogenic microbe has penetrated does not necessarily have to succumb to the disease. For this certain conditions are essential. First, the organism must be susceptible to the disease and the pathogen itself must be sufficiently virulent.

For instance, anthrax bacilli recently extracted from a dead body are highly virulent, they are capable of causing the disease in its gravest form. A rabbit inoculated with a very small dose of the bacilli contracts the disease and dies. But if the same bacilli cultivated in a culture medium are left in the laboratory for several months (without change of the medium) they will cause a rabbit no harm even if introduced subcutaneously in much larger quantities. Consequently, if conditions are unfavourable, the virulence of anthrax bacilli diminishes.

Whether an infectious disease is contracted or not largely depends on the resistance of the macro-organism (the organism of man or animal), i.e., on the degree of its natural immunity, especially at the time of infection.

Fowls are naturally immune to anthrax; a chicken in-

oculated with anthrax bacilli will not contract the disease. In order to undermine its natural immunity, Pasteur stood a chicken in cold water, and then the infection proved effective. Another example: the tubercle bacillus has entered the lungs of a child. If the organism is sound and its resistance high, the child does not contract the disease and, on the contrary, if the child's organism has been enfeebled by some disease, say, whooping-cough, its resistance is lowered and the child may become infected.

The environment—the conditions in which an organism exists—greatly influences its resistance to infectious diseases. An epidemic of typhus raged in Soviet Russia during the civil war. This was due above all to the very difficult conditions in the country: to the famine and cold in the towns, the filth and crowded conditions on the railways. All this, added to the inadequacy of the anti-epidemic measures, caused typhus to spread far and wide. After the civil war, when living conditions improved and sanitation was organized on a much larger scale, the epidemics of typhus and other infectious diseases were stopped.

* * *

Sometimes the conditions for the development of a pathogenic microbe in a human organism may be quite favourable, and yet the organism will remain healthy. Such people, although healthy, are bacillus-carriers. This usually happens to those who have recovered from a disease. Sometimes typhoid-fever or dysentery bacteria remain in the organism long after a person has recovered from the disease; the bacteria are especially numerous during the period of convalescence. They occur in great numbers in his faeces. The normal period of carrying in convalescents (and after recovery) is 2-3 weeks, but sometimes it lasts for several months and even years.

By excreting microbes into the environment bacillus-carriers may infect healthy people. Bacillus-carriers

(diphtheria, for example) are a grave danger to society, particularly if they happen to be employed in a kindergarten, nursery, or other children's institution, because they spread droplet infection when speaking, coughing or sneezing. Persons carrying bacilli of typhoid fever or dysentery are liable to spread the disease if they work as cooks or scullery-maids in dining-halls, restaurants, children's food-dispensing centres, or as shop-assistants in grocery shops, and other institutions dealing with the preparation and distribution of food. Therefore, in the Soviet Union anyone working in a children's or public catering institution undergoes a bacteriological test before starting work and at stated intervals during work.

Pathogenic microbes may spread through direct contact, as for instance, gonococcus, the organism of gonorrhoea, which spreads through sexual intercourse.

Intestinal diseases may develop from eating with dirty hands and from touching things contaminated by sick people. It is easy, for instance, to catch cholera, typhoid fever or dysentery by touching the clothing of patients.

Humans can catch infections from other humans and from animals. A man can contract anthrax when skinning a horse that died of this disease, or plague from rodents, as has sometimes happened to *tarbagan* hunters.

People can become infected from water in which the cholera vibrio, the typhoid-fever organism, and other pathogens are present. Finally, human beings can become infected by intermediate agents such as the anopheles, carrying in its body malaria parasites, or the louse, infected with typhus rickettsiae, etc.

Each particular pathogen causes a specific disease in man or animals, i.e., pathogens are specific.

Some diseases affect only humans, for instance, typhoid fever, dysentery, whooping-cough, etc.

Other diseases spread to man from animals. Thus man becomes infected with glanders from horses, cats and certain other animals.

There are other diseases, which affect only animals and are not dangerous for man, such as dog, hog, and cattle plague, and some other animal diseases.

Some diseases, as for instance, cholera and typhus, are very infectious and spread quickly—this is an epidemic. The outbreak of a disease (for instance, anthrax) among animals is known as epizootic.

Localities where a certain disease is recurrent—irrespective of whether it is cholera, plague, or malaria—are called endemic centres of the disease.

Infections spread also when minute droplets escape from a sick person at sneezing and coughing. A characteristic example of "droplet infection" is influenza.

When a flu patient coughs or sneezes, myriads of microscopic droplets fly from his mouth and are carried to people around him; the droplets containing influenza pathogens settle on the mucous membrane of the nose or mouth of healthy persons who become infected and may in their turn infect others. That is how a flu epidemic breaks out; diphtheria, whooping-cough, tuberculosis and some other diseases spread in a similar way.

INTESTINAL DISEASES

Typhoid Fever and Dysentery

Hot summer season brings large numbers of flies. They can be seen everywhere—on food remains, soiled linen, garbage, in privies.

In search of food flies settle on excrements, which may contain the faeces of people ill of dysentery and typhoid fever. Bacteria causing these diseases become attached to the legs and get into the intestinal tract of the flies, which settle on food and thus carry the bacteria. People who eat polluted food develop the diseases.

Bacteria, especially the *Bacillus typhosus*, may contaminate water and milk which become the channels

through which the diseases spread and cause "water" or "milk" outbreaks.

Contagious dysentery has been known for thousands of years. Epidemics of dysentery often occurred during wars: thus all the wars beginning with the 15th century and up to World War I were accompanied by outbreaks of dysentery. Sometimes the disease accounted for more casualties than enemy fire. A severe epidemic of dysentery spread among the troops of the Austro-Prussian coalition fighting against France's revolutionary forces in 1792. The French called the disease "Prussian diarrhoea."

The causal organisms of typhoid fever and dysentery were discovered in the late 19th century.

To prevent the spread of the diseases, it is imperative to destroy flies. Garbage, the breeding ground of flies, should be kept in closed bins, and the bins themselves, as well as cesspits and public privies, etc., should be sprinkled with DDT, hexachlorane, and the like. Maximal cleanliness must be attained and water must be effectively protected from pollution by patients' excreta.

A good and widely practised way of protecting the population from infectious intestinal diseases is vaccination which creates artificial immunity.

Active immunization against typhoid fever is produced by anti-typhoid inoculation.

The vaccine contains per cubic centimetre 1,000,000,000 typhoid organisms killed by heating or by formalin.

Vaccination today is commonly done with what is known as the triple vaccine, containing 1,000,000,000 typhoid, 250,000,000 para A, and 250,000,000 para B organisms in cubic centimetre. This vaccine (containing killed organisms) is injected subcutaneously three times at intervals of 7, 10 and 15 days.

The vaccination provides immunity for one year; then revaccination is administered (2 injections) followed by another revaccination (one injection) twelve months later.

Vaccination is often accompanied by a rise in temperature, a general feebleness and an inflammation around the injection. This reaction is not dangerous, it means that, since the introduction into the human organism of a small number of killed bacteria has caused a mild form of the disease, as it were, the individual would be in grave danger if he happened to catch the infection without previous inoculation. As a rule, those who have been inoculated do not contract the disease, or if they do, it takes a mild course.

Sometimes two strains of killed dysentery bacteria are added to the triple vaccine, so as to minimize the number of injections. This is the pentavaccine, containing the typhoid, para A, para B and two kinds of dysentery organisms.

ASIATIC CHOLERA

Cholera is endemic in Bengal, India, in the lower reaches and the delta of the Ganges. That is why it is called "Asiatic." India has been the centre of cholera for ages: when Alexander the Great approached the borders of India and learned that a severe disease (probably cholera) was raging in the country, he turned back.

Up to the time of World War II India was a British colony and the overwhelming mass of the population lived in dire poverty, so that sanitation and hygiene were not even dreamed of.

The warm climate of the lower reaches and delta of the Ganges with a mean temperature of 77°-85°F. affords excellent conditions for the cholera vibrio.

During the period of tropical rains the river overflows and floods the surrounding country. For safety's sake the people built their houses on tall earthen mounds beside which were deep pits. At high water the pits filled with water, in which the women washed their linen, including that of the cholera sick. These pits received sewage and

the polluted waters from the primitive privies and also provided the drinking water for the population. This was the main reason why cholera epidemics broke out so often in India.

Cholera had already visited Arabia and neighbouring countries in ancient times. But the lack of convenient roads and the slow rate of travelling in those days prevented the spread of this fatal disease.

That was probably why Europe had been free of cholera until the 19th century, for before a sick person could reach Europe he either died, or recovered and ceased to be a bacillus-carrier. But as soon as the ways of communications improved cholera came to Europe.

Beginning with the 19th century cholera outbreaks in tsarist Russia occurred almost every other year. Only a few years before the Great October Revolution, in 1908 and 1909, cholera persisted in Petersburg, because the water supply in the city was contaminated.

Old people remember to this day what horror cholera epidemics used to instil in people. As in the case of plague, the authorities avoided calling cholera by its proper name. They called it "common diarrhoea," although during the Crimean War of 1855 about 40 per cent of the "diarrhoea" patients died.

The causal organism of Asiatic cholera is the vibrio in the shape of a comma (see Chapter II).

When the vibrio enters a human organism in food or water, it rapidly proliferates and poisons the organism with its exotoxins. The disease starts with griping stomach pains, and excretion of a peculiar watery fluid, like rice-water. The patient loses a large quantity of liquid matter and seems to dry up. The skin becomes bluish, wrinkled and cold. Violent cramps occur, the temperature falls to 93.2°F. and almost half the cases die in 2 or 3 days.

Protective anti-cholera vaccination is a reliable method of preventing infection.

Cholera vaccine contains 4,000,000,000 organisms per cubic centimetre and is injected three times at intervals of 7-10 days.

When there is a danger of a cholera epidemic, in addition to vaccination, other prophylactic measures are necessary. The use of unboiled water must be ruled out, fruit and vegetables should be eaten cooked, bread should be toasted and, most important of all, hands must be washed often and thoroughly.

Cholera does not occur in the Soviet Union and the people know very little about this dreaded disease.

BRUCELLOSIS

Between Sicily and the North African coastline in the Mediterranean lies the Island of Malta. In mid-nineteenth century the British garrison on the island suffered from a wasting fever and many men were incapacitated. In some cases the disease resembled typhoid fever, in others it dragged on for several months, alternating with apparent recovery, and sometimes the patients suffered from acute pain in the joints.

The disease was first described by Hippocrates and in the 18th century by many other learned men, who called it Malta, Gibraltar, Crete, Neapolitan, or simply Mediterranean fever, because it occurred in that area.

Robert David Bruce, a British bacteriologist, undertook a persistent search for the causal germ of the fever in the eighties of the past century. His numerous investigations led to the discovery of a micrococcus in the spleen of a soldier who died of the fever. Bruce named it the *Micrococcus melitensis*. About 20 years later goats in the Malta were discovered to suffer from the same disease.

The cause of the Malta fever was thus traced to goats. Army doctors forbade soldiers on the island to drink raw goats' milk, and after that no new cases were reported.

Eventually it was proved that the Malta fever was not confined to the Mediterranean, it occurred in a number of other countries. Besides goats, the disease was found to affect cows, sheep, swine, camels, dogs, rabbits, Siberian marmots, and other animals. Cows, ewes and sows suffering from the disease (which was named brucellosis, after the discoverer of the germ) usually slip; this is known as contagious abortion.

Hence, brucellosis is not only injurious to man, but is a veritable scourge of animal husbandry, as it kills the unborn young.

A later discovery showed that people may become infected with brucellosis not only in localities where there are diseased animals, but far from such centres—through eating insufficiently salted meat, or, especially, Bessarabian cheese in which the germ can live and preserve its virulence for 2 or 3 months. Not only raw milk of diseased animals, but butter, cheese and other dairy produce prepared from such milk, are dangerous.

Soviet doctors practise protective anti-brucellosis vaccinations.

In localities with a high incidence of brucellosis among livestock, anti-brucellosis vaccination of animals has proved highly effective. People who run the risk of infection—the staffs of cattle-breeding farms, brucellosis infirmaries, meat-packing plants, etc.—are also vaccinated.

Anti-brucellosis vaccine contains live but weakened bacteria, which are incapable of causing the disease but at the same time create immunity. Vaccination is administered once a year, but only to healthy individuals. Special analyses are employed to establish that the person to be vaccinated has not got the disease.

Brucellosis is an exceedingly dangerous and wasting disease; therefore vaccination should be encouraged in every possible way.

This happened in a small Dutch town in 1895. A group of 34 musicians held a memorial feast at which they ate to their hearts' content. The next day 20, who had eaten ham, were taken seriously ill and three died. The doctors were sure it was food-poisoning.

Van Ermengem, a Dutch scientist, made a thorough bacteriological examination and concluded that the poisoning was due to an anaerobic bacterium, which he named *Clostridium botulinum*,* because similar food-poisoning had occurred previously and was often associated with eating sausage, ham, etc.

The first descriptions of the disease, dating back to the beginning of the 19th century, were made by Zengbush in Russia (1818) and J. Kerner, a poet and physician who lived in Nuremberg in 1820.

Further research showed that botulism was caused not only by eating sausage, but also by eating fish (especially such as sturgeon, *beluga*) and canned vegetables and meats.

This is due to the fact that the botulinum bacillus forms spores which possess a high thermal resistance: they are killed only by heating for 20-30 minutes at a temperature of 239°-248°F.

The product of botulinum bacillus is a toxin of exceptional potency, the minutest dose of which is sufficient to cause death.

A Russian scientist, Anrep, discovered the toxin in 1885. The bacillus is anaerobic and often occurs in soil. Vegetables and any other product coming in contact with soil may be polluted with the clostridium or its spores which penetrate to the intestinal tract of animals and fishes.

The botulinum organism may enter fish either through the alimentary canal or through wounds inflicted by

* From the Latin *botulus*—sausage.

fishing tackle. This has been established by Soviet scientists Konstansov, Arustamov, Burova and the workers of the Erisman Institute of Sanitation. The botulinum toxin begins to show its effect 12 to 24 hours after its penetration into the human organism with food. It affects the medulla oblongata, the spinal cord and the vision. Grave cases of botulism end in death from heart paralysis.

The only means of saving people who have developed botulism is the injection of anti-botulinic anti-toxic serum as early as possible. If time is lost the serum will prove of no avail and the patient will die. Anti-botulinic serum must also be administered to people who are not ill, but have eaten products known to contain the toxin.

SMALLPOX OR VARIOLA

Every citizen of the Soviet Union is vaccinated against smallpox at an early age and usually bears on his arm a round or oval mark. Vaccination protects children from smallpox, and our young people have a very vague notion of this grave disease. On April 10, 1919, Lenin signed a law making it compulsory for all citizens of our country to be vaccinated against smallpox. This led to the gradual disappearance of the disease which used to be humanity's scourge. Even now it occurs in countries where vaccination is not compulsory.

It is hard to tell when smallpox appeared. Its origin is lost in antiquity. It was known to exist several thousand years B.C. in Africa, India and China. The Arabs who overran Europe in the 7th century brought smallpox with them which became so widespread that in the 16th and 17th centuries there was hardly a person whose face did not bear its traces. But that was not all the harm it caused: the disease took a heavy toll of life and in Germany alone the annual smallpox mortality was 70,000. In France the death-rate was lower—up to 30,000—but

almost a quarter of the population were pock-marked and many were blinded.

With the discovery of America smallpox was introduced into that continent. Almost the entire local population suffered from smallpox and mortality was very high.

In Europe the situation was better, and yet during the last three centuries smallpox carried away not less than 150,000,000.

Smallpox is caused by Paschen bodies, germs passing through large-porous bacterial filters. The causal agent of smallpox is therefore grouped with filter-passing viruses.*

Enormous numbers of the virus accumulate on the mucous membrane of the nose and larynx of smallpox patients who cough almost incessantly. The virus is discharged from the sick person in droplets of sputum. Healthy people are infected with smallpox mainly through these droplets, but direct contact with contaminated objects or corpses is also infectious.

The skin on the face, arms, hands and legs of a person suffering from smallpox becomes covered with watery blisters which in the course of two or three days fill with pus. On the 11th or 12th day the eruptions begin to dry up and cause intense itching. After 30-40 days crusts drop off the pustules, leaving pock-marks which remain for life. Sometimes, however, smallpox causes blindness and deafness, and 10 to 30 per cent of the cases prove fatal. During an outbreak of black pox hardly any one survives.

Besides the smallpox of humans there are cowpox, sheep-pox, ass-pox, and even bird-pox. But the animal forms are not dangerous for man and cause only local lesions, which has enabled us to use cowpox for protective vaccination. The subject will be dealt with at greater length in the next chapter.

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* Another important property of filtrable viruses, including the Paschen corpuscles, is that they exist only as parasites in tissues of man, animals and plants.

Now that important progress has been made in the treatment of diphtheria it is no longer the dangerous and fatal disease it used to be 50 or 60 years ago.

In his *Sur l'eau* Maupassant described the terrible pain and torments of those who were dying of diphtheria, suffocating from the membranes in their throats.

What could doctors do at that time but try to remove one of the membranes? There was a temporary abatement of the patient's sufferings, and then suffocation set in again, and the patient died.

Maupassant went to a farm where he found the mother and her daughter dying of diphtheria; the father and son had died before them, so that the disease had wiped off the whole family. In the 19th century the picture described in the story was common enough.

Humanity suffered from diphtheria for many centuries. From the 15th to the 17th centuries the disease was described in detail by Spanish doctors who called it *garrotillo* (the strangler's noose), a name that was preserved for a long time.

Diphtheria became especially widespread in the 19th century when it penetrated to all corners of the globe, reaching Australia and the Pacific islands. The means of combating diphtheria was discovered at the end of that century (see Chapter XI).

The causative organism of diphtheria is a bacterium in the shape of dumb-bells (Fig. 41).

Thickenings are sometimes discernible not only at the ends but in other parts of the bacterium, which was identified in 1883-84 by Koch's pupils Klebs and Loeffler. The bacteria elaborate a potent toxin which kills children, as has been convincingly established by Roux, a pupil of Pasteur's.

Diphtheria bacteria are highly resistant to drying and other unfavourable conditions; they have been discovered

on perfectly dry objects such as toy cubes a sick child had played with three months before.

Diphtheria disseminates mostly through droplets, but toys, books and other objects handled by carriers, are also infectious. Settling on the mucous membrane of the nose or pharynx the bacteria multiply, producing a toxin which poisons the organism. On the spot where the bacteria grow there appear inflammation and tough membranes of a dirty-white or greyish colour. The disease derives its name from the Greek *diphthera*—leather. The accumulated necrotic membranes, securely connected to the adjoining tissues, cause asphyxiation. In grave cases of diphtheria death occurs as a result of general toxaemia.

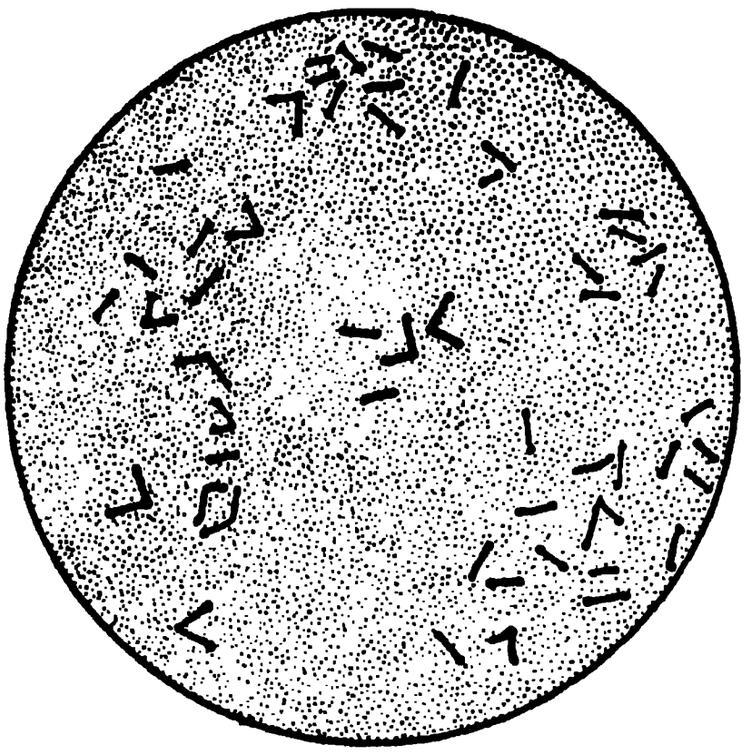

Fig. 41 Diphtheria bacteria

Today diphtheria toxin which can be lethal to man is utilized in a modified form for prophylactic purposes (diphtheria anatoxin), and an antitoxic serum is administered for treatment.*

All children are vaccinated against diphtheria with diphtheria anatoxin before they are one year old: after the first vaccination, the second is administered at an interval of 20 days, and three or six months later—the third. Vaccination is repeated at the age of 4 and once more between the ages of 8 and 12.

* See Chapter XI, p. 205.

The means of preventing and treating diphtheria at the disposal of Soviet doctors are highly effective and diphtheria epidemics are unknown in the U.S.S.R.

TYPHUS

As has been said in Chapter II, typhus is caused by rickettsiae. C. Nicolle, a French scientist, revived the idea of the Russian scientists G. Minch and N. Gamaleya by proving that typhus rickettsiae are transmitted from a sick man to a healthy person by lice.

Feeding on a person ill of typhus a louse sucks his blood containing rickettsiae which multiply in its intestinal tract for 4-6 days and are then excreted. Biting a healthy individual the louse leaves its excreta near the bitten spot. In scratching the bite the healthy person rubs the contaminated excreta into his blood. Infection may also occur if a louse containing rickettsiae is crushed on the body of a healthy person.

When there is an epidemic, the best protection against typhus is to treat linen with substances killing and repelling lice, such as "K" soap, DDT, hexachlorane, etc.

Scientists hold that the linen of typhus patients is also infectious, since it may carry the excreta of typhus lice.

What are the symptoms of typhus and the course of the disease?

Between the moment a rickettsia-carrying louse has bitten a healthy man and the first manifestation of the disease there intervenes a period of from 11 to 14 days. The temperature rises to 103.2°F., and even higher. The patient is feverish and feels very weak. The face is red, there is a racking headache. On the 4th or 5th day an eruption appears on the body. The activity of the heart and blood vessels is disturbed. If the patient's organism has successfully coped with the disease the temperature begins to fall rapidly on the 14th-15th day, becoming normal in two or three days.

But it is not every organism that can overcome the typhus rickettsia. In patients with a weakened heart, old people and drunkards the disease takes a severe course, and often terminates in death.

The application of antibiotics has greatly reduced the mortality from typhus.

RECURRENT (RELAPSING) FEVER

The name of the disease, first described by the Russian doctor Z. Govorlivy in 1861, aptly reflects its nature. A patient has fever for 5 to 7 days. Then the temperature becomes normal. In 5-10-12 days the temperature rises again and remains so for several days, falling once more, only to rise anew after a certain number of days.

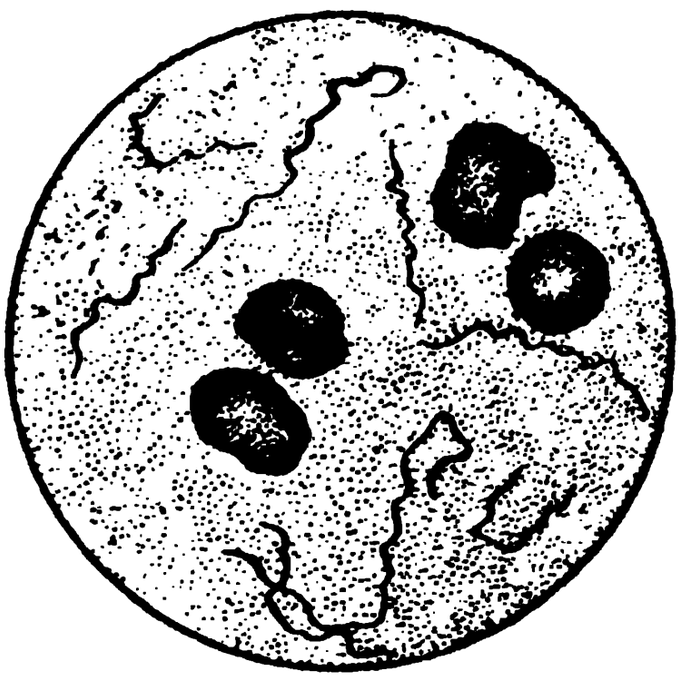

Fig. 42 The Obermeier spirochaetae, the causative organisms of recurrent fever

Spirochaete, the causal organism of relapsing fever (Fig. 42), was discovered in 1873 by the German scientist O. Obermeier. But he died three months later, before he could study its properties.

The study was taken up in Russia. In No. 1 of the journal *The Medical Courier of Moscow* for 1874 G. Minch showed that the microbe identified by Obermeier, which Minch called a spirillum, was the causative organism of relapsing fever.

Further research showed that the disease known as epidemic relapsing fever was also transmitted by lice.

Prof Latyshev in his field laboratory

Besides epidemic relapsing fever, there is endemic relapsing fever in which the spirochaetae are transmitted by wood ticks. The symptoms and the course of the disease are almost identical with those of epidemic relapsing fever.

Tick typhus has been well studied by Russian scientists. One of them, N. Latyshev, inoculated himself with the disease, from which he, fortunately, recovered.

Tick typhus is widespread in Africa, India, North and South America, Spain, Persia, Asia Minor, the Caucasus and Central Asia.

INFECTIONS BY PYOGENIC MICRO-ORGANISMS

Sometimes pimples or boils appear on the skin of man —these are furuncles which are not always easy to get rid of. No sooner has one disappeared than another appears on some other place. Furuncles may appear in

colonies—in the armpit, on the neck, etc.—this is furunculosis.

Boils are caused by staphylococci.

It was Louis Pasteur who identified staphylococcus in 1880 after he had isolated it from the pus of a furuncle. Most of the numerous strains of staphylococci are harmless saprophytes, pathogenic properties occurring among the *aureus* and *albus* strains.

Pathogenic staphylococci are widespread in Nature and are almost always present on human skin, penetrating into every lesion. That is why pus appears in all wounds that have not been properly treated.

In the rare cases when staphylococci invade large blood-vessels septicaemia sets in, which may prove fatal.

* * *

Puerperal sepsis, commonly known as childbed fever, used to be the scourge of all maternity homes in mid-nineteenth century. Over 20 per cent of all child-births were accompanied by septicaemia and death of the mother.

The disease caused such havoc that sometimes it necessitated the closing of homes. This measure, however, was of little avail.

Pasteur discovered the cause of childbed fever, which was cocci arranged in chains, hence the name streptococci (from Greek *streptos*—chain). It was established later that streptococcus species are very numerous and, for the most part, harmless. But there are pathogenic streptococci responsible for septicaemia, septic sore throat, erysipelas, various local infections, including phlegmonosis, and many other maladies. With streptococci universally occurring in great numbers, it was not surprising that so many women died in child-birth. At the time described in Chapter I surgery and obstetrics knew no antiseptic or aseptic, and no measures were

taken to prevent injurious bacteria from penetrating into the patient's blood.

Since medicine mastered methods of combating pathogenic microbes by introducing aseptic and antiseptic, i.e., preserving the wounds from infection, destroying pathogens with physical and chemical agents, cases of septicaemia and similar infections have become extremely rare.

With the invention of penicillin and other antibiotics such infections have become still less dangerous. Given a timely treatment, streptococcus or staphylococcus infection is arrested and the patient's recovery ensured.

FOREST-SPRING ENCEPHALITIS

In 1932 doctors working in Ussuri Territory in the Far East came up against a new and devastating taiga disease.

Often people who returned from the forest developed an acute fever, followed by symptoms of brain disturbances, paralysis of the neck, arms and legs, failure of speech and swallowing. The disease was often fatal. Nearly a third of cases were lethal, and those who survived became invalids for life.

A head drooping on one side and either paralyzed or unwieldy hands are the characteristic signs of encephalitis. In 1935 Dr. A. Panov expressed the view that the disease was Japanese encephalitis, but neither he nor any other doctor could prove it.

Meanwhile spring and summer brought new victims of the taiga disease.

In 1937 a scientific expedition headed by Prof. L. Zilber was sent to the Far East from Moscow. The expedition was well equipped with all that might be necessary for its work, but its main asset was the enthusiasm of its members—Prof. Zilber, M. Chumakov, E. Levkovich, A. Shubladze, V. Solovyov and others.

For months they sought the mysterious and dangerous foe lurking in the forest and finally tracked him down. Prof. Zilber and his co-workers established that it was encephalitis, a disease of the brain, but the form was new, not the one that was known as Japanese. They also established that the disease was contracted in the spring and summer by people who went to the taiga. They discovered the agent, a filter-passing virus, which, introduced into the blood of man by some insect presumably transmitting the virus, causes the disease.

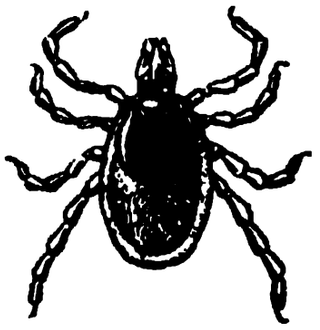

Fig. 43 The *Ixodes persulcatus* tick, the transmitter of the spring encephalitis organism (natural size 3 millimetres)

All this was valuable knowledge, but the triumph of the expedition was marred by the illness of M. Chumakov. He contracted encephalitis and was saved by the blood serum of another member who had had the disease previously. V. Solovyov and E. Gnevisheva, the laboratory assistant, also survived encephalitis.

But the expedition did not provide an answer to the question: who was the transmitter of the spring encephalitis organism? Or in other words: where was the reservoir of the virus? One warm season was not enough to find an answer to this question.

Several other expeditions under the joint guidance of Academician E. Pavlovsky and Prof. A. Smorodintsev were subsequently sent to solve this difficult problem. Finally the transmitter was discovered: the tick *Ixodes persulcatus* (Fig. 43), which abounds in the taiga along pathways frequented by men and animals and in spring and early summer attacks man in great multitudes, because then the number of ticks in the forest is the greatest.

How does the tick become the transmitter of the virus? Academician Pavlovsky's expeditions worked hard and

persistently until they were able to prove that the reservoir of the virus are the chipmunk, a Far-East rodent (Fig. 44), and the field-mouse. Squirrels, hares, hedge-

Fig. 44 Chipmunk, the animal reservoir of the spring encephalitis organism

hogs, moles, and even thrushes and hazel-grouse can be infected with the forest encephalitis virus.

The minute three-millimetre-long tick parasitic on diseased animals and birds sucks their blood, with which the virus enters its body, and by biting man and healthy animals and birds, infects them with encephalitis, thus increasing the number of sources of infection.

The Soviet research workers paid dearly for this knowledge: parasitologist B. Pomerantsev, a member of Academician Pavlovsky's expedition engaged in the study of the transmitters, contracted encephalitis and died.

Further research established that persons who had survived forest encephalitis acquired immunity for life. But it was necessary to create the immunity artificially,

to invent a vaccine which would not endanger the life of man and ensure immunity for the future.

N. Kagan has succeeded in preparing a vaccine from the brain of white mice affected with encephalitis. N. Kagan and her assistant N. Utkina worked on highly infectious material and the vaccine they prepared proved very effective, but insufficient experience in handling the new virus proved fatal to the two courageous women.

In 1939-40 E. Levkovich and A. Smorodintsev tried the vaccine on themselves and corroborated its effectiveness. After that the local population of the taiga region was vaccinated with very good results. Out of a thousand vaccinated persons only two contracted the disease at a later date and had it in a very mild form, whereas out of a thousand unvaccinated persons 26 developed the disease and 8 cases out of the 26 proved fatal.

PLAGUE

The word "plague" is derived from the Latin *plaga*—a blow.

Although in the historical survey we spoke of plague epidemics up to 1770-72, this by no means implies that there were no epidemics at a later date. True, the 19th century was relatively free from plague epidemics, but outbreaks occurred in the Volga region, in Stavropol, Odessa, the Crimea and the Transcaucasus (a violent outbreak in Georgia in 1840) in Russia, as well as in other countries.

In mid-nineteenth century plague was stamped out completely in Europe, but persisted in other continents. Thus an outbreak of plague occurred in Hong Kong in 1894 and it was there that the examination of corpses led to the discovery of the causal organism, a minute egg-shaped bacterium, whose ends are well stained by aniline dyes (Fig. 45).

A series of plague epidemics started in 1896 in British India where tens and hundreds of thousands of Indians died every year, the figure in some years exceeding a

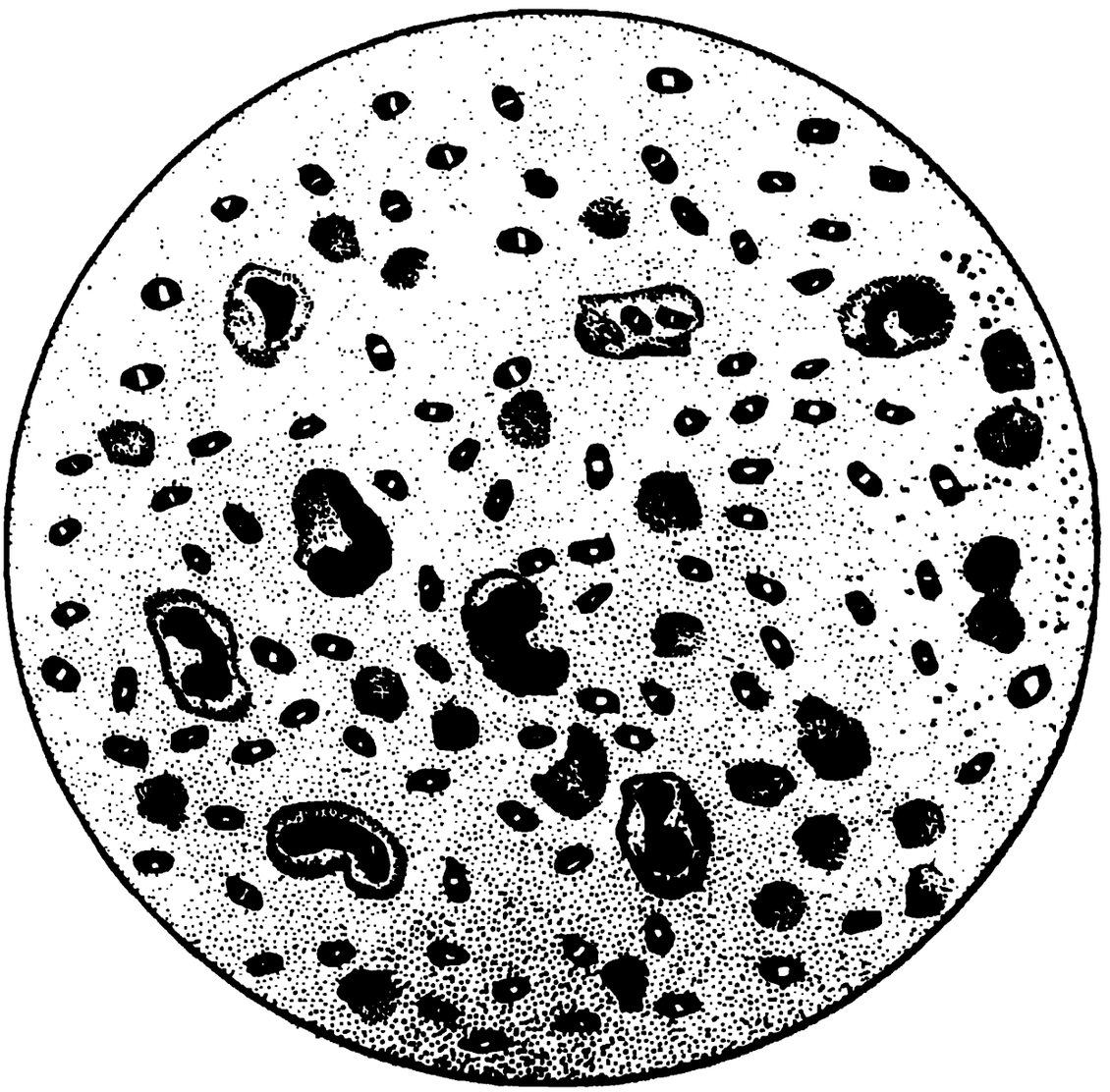

Fig 45 Plague bacilli

million (e.g., 1904-05 when the number of plague victims was 1,328,249).

Outbreaks occurred in Manchuria, where the 1910-11 epidemic of pulmonary plague was especially severe.

In spite of the fact that humanity knows how to protect itself against plague, it still exists, particularly in Asia where over 26,000 persons were stricken by plague in 1938. The reason is that there are reservoirs harbour-

ing plague microbes—rodents susceptible to the disease such as Siberian marmots, *tarbagans*, rats, etc. (Fig. 46).

The disease is transmitted from the animals to man by various means, especially by fleas who have sucked up the blood of diseased rodents. Plague-affected rats infect homes, ships, etc. Men bitten by plague-carrying insects usually develop the form of plague known as bubonic, but sometimes rodents transmit the lung form. We owe our present knowledge to the selfless work of Russian scientists D. Zabolotny, I. Deminsky and their colleagues.

The plague bacillus enters through the human skin and along the lymphatic vessels penetrates to the glands (the inguinal, axillar, cervical, and other) where inflammation sets in. The glands swell, forming buboes, from which this form of plague derives its name.

The human organism is an excellent medium for the bacilli's growth, and countless germs invade all the organs and the blood-vessels, causing acute intoxication of the entire organism. The disease develops very quickly and in a few days the patient dies of heart paralysis.

Fig. 46 Tarbagan, the animal reservoir of plague

When the plague bacteria invade the lungs the disease takes the form of pneumonic plague. A person affected with pneumonic plague coughs up blood in sputum containing myriads of plague bacilli; such patients disseminate the bacteria and spread droplet infection to those around them.

Until recently mortality from bubonic plague was between 40 and 90 per cent, and from pulmonary plague 100 per cent. A diagnosis of "pulmonary plague" was tantamount to a death sentence.

Plague has been completely eliminated in the Soviet Union, but in foreign countries outbreaks of plague, including the pneumonic form, still take place.

Soviet scientists are keenly interested in protecting humanity from pulmonary plague. The persistent researches that have been conducted by N. Zhukov-Verezhnikov and his co-workers have resulted in the discovery of a remedy against the disease. This remedy has proved very effective in treating cases of pulmonary plague. In addition, if the danger of a plague outbreak arises, preventive vaccination is practised with a vaccine containing live but greatly weakened plague organisms.

ANTHRAX

Anthrax has been described as one of the "Egyptian plagues" of ancient times. It was known in India 1,000 years B.C. Homer, who lived in the 9th century B.C., wrote in the *Iliad* of a "sacred fire" (anthrax) striking both animals and men. Plutarch says in his works that anthrax spread among animals and men so quickly that it was impossible to define the moment the illness set in.

Anthrax was widespread in ancient Italy, and later its endemic centres appeared in all European countries. In Oriental countries anthrax was known as "Persian fire."

We do not know exactly when anthrax penetrated to Russia but a disease known as "fire disease," "Siberian disease" and "Siberian sore" broke out in 1738-39 in a military settlement on the Gryaznukha River, South Siberia, where many people and animals died of it. G. Miller, a Russian historian, gives a detailed description of the epidemic and epizootic.

Daniil Zabolotny

Anthrax acquired its Russian name of "Siberian sore," because in the subsequent years of the 18th century and in the 19th century the disease affected mainly certain localities of Siberia.

In his monograph on anthrax published in 1865 in Odessa, T. Loginov says: "I remember that between 1798 and 1802 at the Urals works beyond the mountain range, that is, in Siberia, a disease had broken out in some localities, which was then called malignant pustule and which affected cattle, horses, and even men."

Here is what F. Shkinsky, an army doctor, wrote in his *Siberian Fever*, published in St. Petersburg in 1832: "The authorities have many reports from doctors, besides this one, regarding the frequent appearance, or rather, permanent existence, of this malignant fever in many provinces of Siberia."

In 1864 an epizootic again broke out among cattle in Siberia. The illiterate peasants, who had various local

I. Deminsky

names for the disease, did not realize its danger. Insufficient veterinary aid and the lack of sanitary education among the population resulted in a high incidence of cases not only among animals but also among humans. Animals died of anthrax in a day or two; men caught the disease from the animals and also died. The epizootic and epidemic lasted two years and carried away scores of thousands of head of cattle and thousands of human lives.

The mortality was especially high in the tundra where great numbers of reindeer died in summer pastures. Between 1896 and 1915 anthrax accounted for the loss of about 500,000 head of reindeer.

The tsarist government took no serious measures against this scourge. After the Great October Revolution, however, numerous veterinaries and doctors were sent to the tundra to carry out effective anti-anthrax measures.

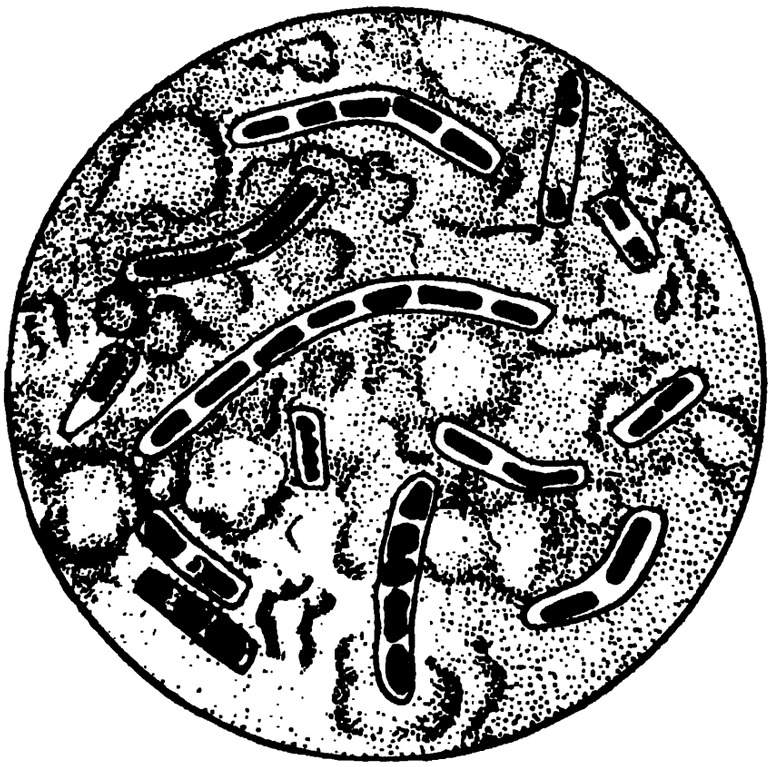

Fig. 47 Anthrax bacilli

Anthrax is caused by big spore-forming bacilli, which look like rods with the ends cut off. On entering an animal or human organism the anthrax bacilli cease to form spores, for here they find optimal conditions for feeding, growing and proliferation. Although the organism fights against the invading bacilli, the fight usually ends in the victory of the latter if the attacked animal has not been vaccinated, or if no treatment is given in time. This is partly due to the fact that in the organism the anthrax bacilli usually form mucous sheath-like capsules which protect them against the attacks of the organism's defence forces (Fig. 47). The bacillus is very dangerous as it is capable of forming spores in the extra-organic media, such as soil, grass, etc., which remain viable and virulent for dozens of years.

Animals which die of anthrax infect the soil and the grass which is then eaten by other animals. Fig. 48 representing the "anthrax circle" shows how numerous are the channels through which anthrax may spread if but one animal has died of this disease. The infection spreads through contaminated soil, grass, any objects that have been in contact with the dead carcass, and

also through a number of anthrax-transmitters, such as horse-flies and gad-flies, birds of prey and rapacious beasts which are infected from diseased or dead animals.

In the old days anthrax was a scourge in some localities, sweeping away horses, bulls, cows and sheep.

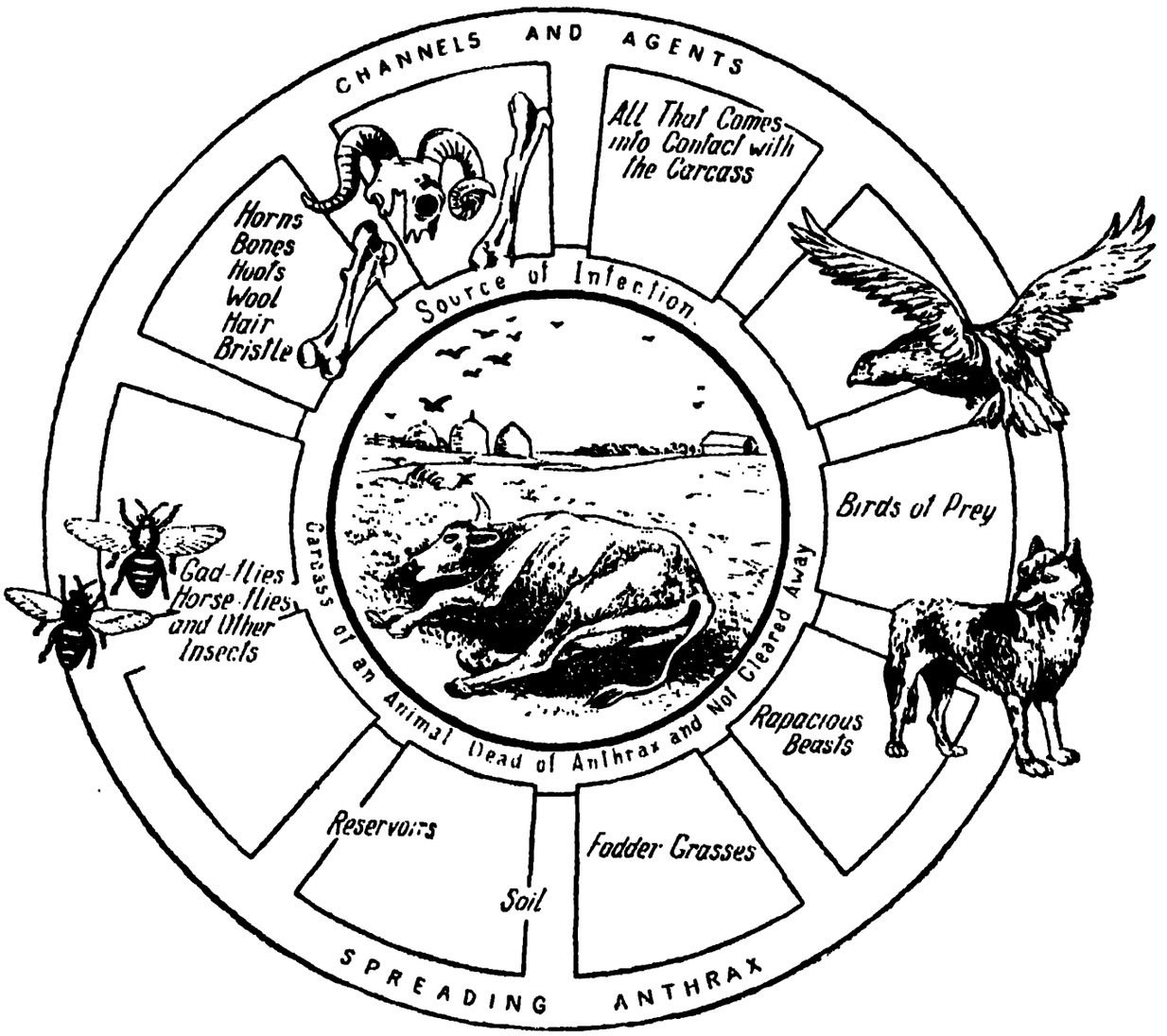

Fig. 48 The Anthrax Circle showing the channels through which anthrax can spread

Shepherds, butchers, flayers, tanners and others who handled animals contracted anthrax even through a slight scratch on their skin.

Men became infected while looking after diseased animals. When an animal died its owner would flay it and use the skin, in which case infection was almost a hundred per cent.

The following episode is characteristic of the tsarist times: during the Russo-Japanese war of 1904-05 merchants supplied the army with sheepskin coats made of the skins of animals which had died of anthrax. This caused numerous cases of anthrax in the army; the epidemic ceased with the spring when the warm coats were discarded.

Workers at tanneries are apt to become infected with anthrax, for skins of affected animals may get into production. Therefore, in the Soviet Union all skins going to factories are previously tested in laboratories.

Modern methods of treatment are very reliable and timely treatment is highly effective. But the ability of the causal organism to form spores makes the disease difficult to eradicate. That is why it still exists in almost all countries of the world, and wherever no strict measures are taken, the percentage of animals affected with anthrax every year is still high. In the Soviet Union all domestic animals are vaccinated against anthrax and as a result there are scarcely any anthrax cases among animals, to say nothing of humans.

TETANUS

Tetanus is caused by an anaerobic bacillus often occurring in soil where it forms spores. Under the microscope the tetanus organisms and their spores look like tennis rackets, the round part being the spores (Fig. 49). Tetanus bacillus was identified in 1884.

A bacteriological analysis of 100 soil samples usually shows at least 25 of them to contain tetanus bacilli. They get into the soil in human and animal excreta, as they occur mostly in the intestinal tract of healthy horses, asses and mules. Sometimes they inhabit the intestinal tract of healthy humans. Tetanus bacilli enter the intestinal tract in food and drinking water, because grass

which animals eat, raw vegetables and water may carry microscopic particles of soil often containing tetanus spores. This is an example of microbial travelling: from the intestinal tract into soil and from the soil back to the intestinal tract.

The main characteristic of the tetanus bacillus is its property to form a toxin of such potency that one-sixty-millionth part of a gramme is enough to kill a mouse.

A minute dose of tetanus toxin is lethal for man, and, somewhat greater, for horse, but not when it enters the intestinal tract, where digestive juices render the toxin harmless.

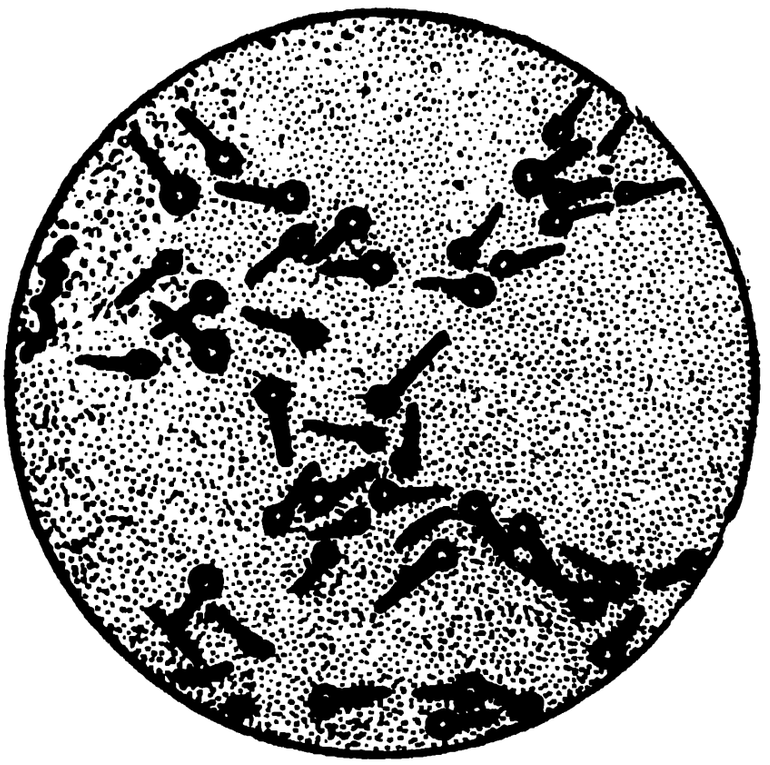

Fig. 49 Tetanus bacilli showing spores

The tetanus bacillus is dangerous for man and for susceptible animals only when it enters a wound. When a navvy or an agricultural worker cuts his hand or foot with his tools (which usually are covered with earth) the bacilli may enter the wound where they multiply and produce their toxin. The toxin reaches the central nervous system by way of the nervous tracts and affects it. The bacilli themselves remain in the wound and do not invade the organism any further.

The poisoning of the nervous system causes violent convulsions and spasms. The body of the suffering man is thrown into such convulsions that he rests only on the back of his head and on the pelvis. The patient dies of asphyxiation and heart paralysis but his mind remains clear.

Cases of tetanus infection during excavations or farm work are comparatively few. This fatal infection used often to occur during wars: the fragments of bombs, shells and hand-grenades which caused injuries nearly always introduced into the wounds particles of soil, and, consequently, also tetanus bacilli which were often present in them. Although at the time of the First World War methods of combating tetanus were known, fatal cases of tetanus infection on the battlefields were fairly numerous, especially on the western front.

A persistent fight is being waged against tetanus in the Soviet Union. Preventive anti-tetanus vaccinations made at the time of the battles at Lake Khasan and Khalkhin-gol, as well as during the Great Patriotic War, prevented tetanic complication of wounds.*

In the U.S.S.R. anti-tetanus vaccinations are administered to workers engaged in excavations and agriculture and also to anyone else who receives scratches during a fall and applies for medical assistance.

RABIES (HYDROPHOBIA)

Sometimes—although not often—a dog in the street attracts general attention by its appearance and behaviour. Its eyes are dull, its tail drooping, saliva is dripping from its open mouth; it may snatch a rag lying on the road, a piece of wood or stone and swallow it. Without the slightest provocation the dog may steal up to a passer-by and bite him, and then run away. A mad dog does not know its master and when called, tries to sneak off. If a mad dog is not caught or killed, it may bite people and dogs, thus spreading the disease. Then the dog is paralyzed and on the fifth or seventh day dies.

* Preventive anti-tetanus vaccinations are made with tetanus anatoxin (see Chapter XI) which is effective for one year. At the end of the year the inoculated persons are re-vaccinated.

Dogs catch the infection from other dogs or rabid wolves which are the chief source of rabies. Besides wolves, dogs and men, cats, rats, mice, some other warm-blooded animals, and even birds, are susceptible to hydrophobia. Cases have been known of humans contracting the disease on being pecked by a rabid bird.

Hydrophobia was known as early as the 4th century B.C. Aristotle mentioned it, and the celebrated Ibn Sina and the Arabian physician Rhazes (10th century A.D.) described the disease in detail.

We know from Chapter I that Pasteur and his predecessors failed to discover the causal organism of rabies. The efforts of many scientists after Pasteur were equally unsuccessful. This is easily explained, for they sought the organism with the help of an ordinary microscope which magnifies microbes not smaller than 0.2 micron. But the causal agent of hydrophobia is a filter-passing virus and, although considered big (100-150 millimicrons), it cannot be discovered by ordinary laboratory methods.

Hydrophobia is usually transmitted by the bite of an infected animal (dog), because the virus is contained in the saliva and enters the bitten spot. Sometimes a mad dog may merely lick the hand of its master and if there is a slight scratch on it, the virus may enter the lesion and infect the man. From the lesion the virus spreads through the body, reaches the central nervous system and when it has proliferated there, the disease begins to manifest itself. The duration of the "incubation" period depends on the distance between the bitten spot and the central nervous system, on the number, size and depth of the lesions, and on the speed with which the virus moves along the nervous system. It may last from 10 days to one year, but usually from 35 to 40 days and, rarely, three months.

What are the symptoms of hydrophobia in man?

The bitten spot begins to ache. The man is unaccount-

ably sad and excited. Sleep is disturbed. When awake the mind wanders. Everything irritates him. A gentle touch, a slight noise produces convulsions. The victim (and this is very characteristic of the disease) fears water: he cannot drink, and the very idea of drinking causes painful spasms in the throat. He is haunted by fear and rushes about seeking to escape some unknown danger. Then the patient begins to rave, he has hallucinations and, later, becomes paralyzed and dies. The disease lasts from 2 to 5 days.

Fortunately, not all that are bitten by mad animals develop the disease. But science is not yet able to determine who has become infected and who has escaped the malady, therefore, if a suspect dog has but licked a hand, the person must go at once to the nearest Pasteur Station, vaccination post or polyclinic to receive a course of preventive vaccinations.

TUBERCULOSIS

The scourge of tuberculosis dates from antiquity. The Code of Laws of Hammurabi, a Babylonian king, compiled 3,800 years ago, contains a description of pulmonary tuberculosis and a clause permitting a man to divorce his wife if she contracts the disease. More than 2,000 years ago, the Code of Manu proclaimed tuberculosis of the lungs an incurable and unclean disease and forbade men to marry women with tuberculosis in the family; so the infectious nature of the disease had already been realized at that time.

As in all countries and at all times, tuberculosis in ancient Egypt affected most often the poor and the oppressed while the upper classes were comparatively free from it.

About 2,550 years ago, Wang Shu-huo, a Chinese scientist, wrote a treatise on medicine, in which he described the unfavourable symptoms of tuberculosis and its treatment.

The Greek writings on medicine and tuberculosis show that ancient doctors considered it a very grave disease; this was probably due to the fact that in those times tuberculosis was diagnosed only at its gravest stage, which usually ended in death. But even then doctors attached great importance to the patient's regimen; they prescribed rest, warm baths, wholesome, but not spiced food, such as milk, etc.

Valuable observations made by ancient Greek doctors were inherited by the Arab doctors of the 10th and 11th centuries and later, by mediaeval Christian medical schools.

The study of corpses has shown what changes are brought about in the human organism by tuberculosis; the tubercles observed in the lungs and sometimes in other organs, have earned the disease its present name.

In 1865 Villemin, a French doctor, proved that the tubercles were infectious and in 1882 R. Koch discovered the causal organism of tuberculosis—the tubercle bacillus.

This bacillus is a short and thin rod encased in a sheath of a fatty and wax-like substance, highly resistant to alcohol, acids and alkalis (Fig. 50). The bacillus resists drying and remains alive in dust, with which it enters the mouth and lungs of man.

Besides man, cattle, birds, monkeys, guinea-pigs, and other animals are susceptible to tuberculosis. Man can contract the disease (especially as a child) from the milk of a tuberculous cow. It is not easy to diagnose tuberculosis in a cow by the usual method of auscultation, and so K. Gelman, a Russian scientist, elaborated the method of obtaining a diagnostic preparation known as tuberculin. A few drops of tuberculin are introduced under the lower eyelid of the cow, or it may be injected intracutaneously. If the animal has tuberculosis, pus will appear in the eye within a few hours, or the spot around the puncture will become swollen.

Tuberculosis is primarily a droplet infection; but man may become infected through the mouth (from infected milk or dust).

When tubercle bacilli enter the lungs where the conditions are favourable, if the resistance of the organism is weakened, tubercles appear around groups of bacilli. Then the tubercles disintegrate producing a caseous mass and pus. When a large number of tubercles thus decompose, large areas of the lung tissue are destroyed and pus-filled caverns are formed.

Fig. 50 Tubercle bacilli in sputum

When tubercle bacilli enter the blood-stream they are carried to all the organs, where they produce tubercles not unlike millet-grains. This is the *miliary* (millet) form. In such cases the process often develops at an exceedingly rapid pace and the man may die in a few months. The disease is known as galloping consumption.

If tubercle bacilli develop in the bone substance this is osseous tuberculosis. When tuberculosis attacks the spine, individual vertebrae become soft, the spine bends and a hump develops.

Lupus is tuberculosis of the skin; it affects mostly the face and the patient becomes disfigured. Like osseous tuberculosis, lupus attacks mainly children.

Tuberculosis is widespread in all human communities, especially where the population is crowded. If people congregate in great numbers, persons ill of tuberculosis

are certain to be among them, and from these the disease spreads to healthy individuals.

That is why many town-dwellers become infected with tuberculosis in early childhood. The entry of the bacillus into the organism does not necessarily result in the person developing tuberculosis; here many factors, especially the individual's living conditions, play an important part.

LEPROSY

From Egyptian papiri we know that leprosy dates from the earliest times.

The researches of G. Minch, a Russian scientist, show that leprosy came to Egypt from India or Persia, and from there spread to other Mediterranean countries. The Phoenicians, who were among the first sea-traders, were instrumental in spreading the disease, and leprosy was often called the Phoenician disease in those times.

After the crusades, when large masses of Europeans went to Asia Minor, where leprosy was widespread, and then returned home, the disease appeared in Europe. The greatest number of lepers was found in European towns between the 11th and 13th centuries, in the heyday of feudalism.

To protect society, all lepers were made to live in leper asylums, or leprosoria.

An acknowledged leper was expelled from society, deprived of the right to inheritance, and his wife was free to divorce him. In some countries funeral services were held for lepers, who were looked upon as dead.

Lepers had to wear special garments with an ace of hearts on chest and back. A leper had to sound a rattle to warn of his approach. The dread in which leprosy was held and the stringent measures taken against the disease considerably lowered its incidence, but in some countries it occurs even now.

G. Hansen, a Norwegian doctor, discovered the causal agent of leprosy in 1871. It is a thin rod-shaped bacillus, resembling the tubercle bacillus in shape and in other respects. Only human beings are susceptible to leprosy, the infection penetrating by way of the skin, the mouth and the nose.

The sores on the face, hands, and other parts of the body teem with bacteria, which also are present in the sputum, and the infection may spread in talking, sneezing and coughing.

The incubation period is very long and may last from 1 to 8 years. Hence an infected individual does not suspect he is ill.

When the disease begins to manifest itself, spots, tubercles and ulcers appear on the skin, particularly on the face. The mucous membrane of the nose and larynx is also affected. The patient suffers from coryza with blood in the discharge; sores appear in the nose and its walls are gradually destroyed. The sores in the larynx cause spasms and the patient suffers from asphyxia. Sometimes sores are formed on the eyes and the patient becomes blind in one or both eyes. The face becomes disfigured. The skin of lepers often loses sensitivity, because in some places the sensory nerves are affected. All the sores on the mucous membrane, hands, face, and wherever else they may be, are inhabited with hosts of bacteria which the leper disseminates in the environment.

Chapter XI

FIGHT AGAINST PATHOGENIC MICROBES

In the preceding chapter we spoke about pathogenic microbes causing infectious diseases in man and animals. How does a human or animal organism protect itself against them?

Attempts to fight infectious diseases were made in ancient times. An original form of vaccination against smallpox was practised in ancient Tibet, India, Persia and in some parts of Africa. Scabs from the pustules of sick persons were dried and crushed, and the powder rubbed into the skin, the nose and scratches on the skin of healthy persons; even snuff was prepared from this powder.

In ancient Georgia variolation was performed by dipping a needle into pustules of a smallpox patient and scratching a healthy person with it. If the person thus vaccinated did not develop the disease at once, he received an immunity against further infection.

Variolation was applied in a number of countries in Europe and elsewhere as late as the end of the 18th century.

E. Jenner, an English doctor, made some very interesting observations in the village where he practised. He noticed pustules on the hands of milkmaids who attended to cows affected with cowpox. The women showed no other symptoms of the disease. In due time the sores dried and scars formed on the hands. Jenner made inquiries and learned that the women who had had cowpox

did not contract smallpox, and if they did, it was in a mild form.

These observations gave Jenner his great idea of vaccinating humans with cowpox.

He studied the problem long and thoroughly, conducting numerous observations and experiments, and in 1796 he finally decided to put his idea to the test.

Sarah Nelmes, a peasant woman, happened to have cowpox and Jenner inoculated a boy, James Phipps by name, with matter from the cowpox vesicles on her hands. A pustule appeared on the vaccinated spot, like the one we witness nowadays. The next step was to see if the boy would be immune against smallpox. In those times the dread disease was a frequent visitor in England and soon it again appeared in the district where Jenner was practising.

To test the effectiveness of the inoculation with cowpox,* Jenner inoculated the boy with matter from a person ill of smallpox. We can well imagine his anxiety during the period of incubation, but at the end of it the disease did not follow. The destructive disease which killed and crippled, proved powerless against the genius of man.

July 1796 marks the beginning of the era of protective vaccination against smallpox. Before the Revolution vaccination in Russia was not compulsory and nearly 100,000 persons had the disease every year; many were blinded or maimed and 15,000 died of smallpox.

In the Soviet Union smallpox has been completely eliminated thanks to compulsory vaccination.

* * *

* Cowpox is caused by a much weaker virus than the one causing smallpox. That is why the virus introduced into the human organism in vaccine (from the Latin *vacca*—cow) does not cause disease but creates immunity to smallpox.

When pathogenic microbes gain access into an organism, there ensues a life-and-death struggle between them and the protective forces of the body. Microbes tend to proliferate very quickly, producing substances harmful to the organism. The macro-organism (human or animal organism) mobilizes an army of phagocytes and antibodies which are produced in the blood and in other organs; the antibodies are specific and destroy specific microbes by weakening them; then they are easily devoured and digested by the phagocytes. Antibodies can also agglutinate microbes, converting them into immovable little lumps easily dissolved by the antibodies.

If the protective forces of an organism are sufficiently numerous, they prevent pathogens from proliferating and destroy them at the outset, checking the progress of the disease. The organism remains healthy.

If, on the other hand, microbes succeed in proliferating, the disease manifests itself and progresses. In such cases the struggle between the aggressors—the pathogenic microbes—and the protective forces of the organism is especially fierce. When these latter are strong enough to overcome the microbe of, say, typhoid fever, the organism recovers at the end, although the disease may take a very grave course. Otherwise, the pathogens invade the organism, multiply very quickly and poison and kill the man (or animal).

Observations have shown that if an individual survives typhus or smallpox he will not usually contract the disease a second time, and if he does, it will be in a very mild form. In other words, having survived some infectious disease, a man (or animal) develops immunity, because, as we have just seen, the organism produces corresponding antibodies.

But it should be borne in mind that if a person has survived one disease (for instance, typhoid fever) the antibodies in his organism protect him from that

disease only. So if that person develops dysentery, his typhoid-fever immunity will not help him to fight dysentery bacteria. This is because the antibodies formed in an organism are highly specific and effective only against the disease for which they were produced.

The immunity we have been discussing is a hard-won one, obtained at the price of illness.

The discoveries of Samoilovich, Jenner and Pasteur have enabled us to create artificial immunity by inoculating weakened or killed pathogenic microbes, the causal organisms of various diseases.

The cowpox virus is much weaker than the smallpox virus and the disease it causes in man is hardly felt. It does not harm the organism in the least, but the protective forces it rouses in it are strong enough to withstand smallpox.

All this held good in the case of animals—sheep and cows—which Pasteur inoculated with weakened cultures of anthrax (the anthrax vaccine). Such inoculations are known as immunization, for they create artificial immunity in the inoculated subjects.

Vaccination with live but weakened microbes prompts the organism to produce large quantities of antibodies, creating a stable immunity. This is active immunity. Pasteur's and Tsenkovsky's anthrax vaccines are an example of inoculation with living microbes.

Today many vaccines, such as typhoid fever, cholera, and some others, are prepared from killed microbes. In order to reduce the number of vaccinations, killed microbes of typhoid fever, paratyphoid, etc., are mixed in a definite proportion; in this way a combined vaccine is prepared, which is effective against several diseases (see Chapter X).

There are also complex vaccines prepared from substances extracted from various microbes. These substances create immunity, just as vaccines prepared with the microbes themselves.

Scientists discovered in the serum of recovered diphtheria patients antibodies capable of neutralizing the toxin of the diphtheria organism. This was called antitoxin.

All children in the Soviet Union undergo compulsory vaccination against diphtheria with diphtheria anatoxin.

What is anatoxin?

If the diphtheria bacterium is grown in a special broth made of pigs' stomachs, the broth contains a very high percentage of diphtheria toxin. The broth is then passed through bacterial filters and thus freed from the bacteria (see Chapter II). A certain quantity of formalin is then added to the broth; the toxin rendered harmless by the action of formalin is anatoxin.

Although non-toxic, anatoxin can nevertheless cause the formation of antibodies in the human organism, which neutralize diphtheria microbes and prevent the development of the disease in case of infection.

If a child falls ill of diphtheria (in cases when it has not been vaccinated, or even in spite of the vaccination) it is treated with another bacteriological preparation—the antitoxic anti-diphtheria serum which contains an antitoxin, neutralizing the toxin produced by the bacteria.

* * *

We have mentioned earlier that tetanus used to be the cause of many deaths, especially in time of war. But since compulsory specific prophylaxis was introduced in the Soviet Union, there have scarcely been any cases of tetanus. The vaccination is done with an anatoxin (prepared just like the diphtheria anatoxin) in combination with other vaccines (the Rogozin method). In addition, wounded men are always treated with anti-tetanic serum.

Food-poisoning with botulinum toxin is treated with an anti-botulinic serum.

We see that humanity has at its disposal various vaccines, sera and anatoxins for preventing and treating many infectious diseases.

A few words in explanation of the difference between vaccines and therapeutic sera.

A vaccine is prepared of the very pathogens (weakened or killed) against which we want the organism to arm itself, or from certain substances extracted out of the microbes. But whether a vaccine is prepared from live microbes, killed microbes or substances extracted from them, it must prompt the organism of man or animal to produce antibodies capable of fighting the infection. The result is the development of active immunity.

When a serum is obtained from an immunized horse, it is used not to prevent infection in the future, but to treat a person or animal already ill, when there is no time to wait for the organism to produce its own antibodies.

Therapeutic and antitoxic sera contain antibodies and antitoxins produced by the horse's organism at immunization. The antibodies and antitoxins fight the pathogenic microbes or neutralize their toxins.

A valuable property of a curative serum is its ability to render immediate and effective aid to the sick person or animal. But the immunity created by sera is passive, lasting for a few weeks only.

Having solved the mystery of the relations between the human (or animal) organism and pathogenic microbes, scientists found effective means of saving them from the baneful action of microbes.

Mechnikov's phagocytosis theory has shed a bright light on the mechanism of immunity and laid the foundation for further development of immunology.

Today the teaching of Ivan Pavlov has enabled Soviet scientists to supplement Mechnikov's theory with the notion that all protective mechanisms of the organism from pathogens are controlled by the nervous system.

Acting on the organism, pathogenic microbes excite the nervous system, especially its highest level—the brain cortex—provoking simple and complex protective reactions of the organism.

* * *

Further penetration into the mysteries of the unseen world has brought one more interesting discovery.

Pasteur noticed that anthrax bacilli throve on broth. But if some putrefactive microbes got in the broth they multiplied so quickly that they oppressed anthrax bacilli and finally destroyed them.

Mechnikov established that organisms of suppuration were in their turn oppressed by lactic bacteria producing lactic acid, harmful to putrefactive bacteria. And this gave him the idea of utilizing microbial antagonism and antibiosis in the interests of human health.

In studying the biological properties of a mould fungus in the seventies of the past century, V. Manasein, a Russian scientist, noticed that if it grew and multiplied in company with some other microscopic fungi, it inhibited their growth. At the same time A. Polotebnov, another Russian scientist, started treating various sores, including syphilitic lesions, with a liniment prepared with bluish-green mould (the penicillium fungus) and almond oil. The results were very good, the sores healed quickly.

But as chemistry was then in its infancy Polotebnov's and Manasein's discoveries were not further elaborated. When, 60 years later, the British scientist A. Fleming noted the wonderful property of the bluish-green mould, his discovery did not bear fruit either; it was only 11 years later, during the Second World War, that scientists, in their search for new remedies, remembered the penicillium fungus.

After several years' work H. Florey and E. Chain evolved methods of obtaining from the *Penicillium notatum* variety a substance inhibiting the development of

staphylococci, meningococci, gonococci and other pathogenic bacteria. The substance was named penicillin.

Soviet scientists worked independently, and Z. Yermolyeva and her co-worker Balezina found in soil another variety, *Penicillium crustosum*, from which they extracted penicillin-crustosin, whose curative properties were in no way inferior to those of American penicillin.

Formerly the lethality of suppurative peritonitis was almost one hundred per cent; the same was true of puerperal sepsis. Thanks to penicillin, these diseases can now be cured. Pneumonia, too, has become less dangerous.

Before penicillin was discovered, cerebrospinal meningitis wards in hospitals were virtually the wards of the doomed (case mortality was 40 per cent). Today hospitals know almost no cases of death from meningitis—owing to penicillin. Scarlet fever, whose case mortality used to be 6 to 9 per cent, has produced no fatal outcomes for a number of years.

Penicillin has proved highly effective in quickly curing gonorrhoea and somewhat less effective in treating syphilis.

The excellent results obtained through the use of penicillin in treating infectious diseases can be explained by the fact that it prevents many kinds of microbes from growing and multiplying. Its action is directed against the life of many pathogenic microbes, that is why penicillin (and substances like it) have the general name of antibiotics.

Extensive researches have resulted in the discovery of another antibiotic—streptomycin, an extract from a strain of ray fungi (actinomyces).

Streptomycin inhibits the development of tubercle bacillus. While formerly an individual who developed tuberculous meningitis was doomed, because the brain covers were invaded by the bacilli, today streptomycin often vanquishes the rod even in the cortical integument and the patient recovers.

Formerly case mortality of pulmonary plague was 100 per cent but now streptomycin in combination with some other drugs can cure the disease. It is effective against tularaemia and some other infections.*

Soviet scientists G. Gauze, M. Brazhnikova and P. Sergiyev isolated from soil a new antagonist, from which they extracted an antibiotic named gramicidin C. It is effectively used in the treatment of sore throat, suppurative processes, some female diseases, the amoebic and bacillary dysentery. In the latter instance gramicidin is applied in conjunction with chemical medicines (sulphamide preparations).

In 1950 Z. Yermolyeva and her co-workers extracted from fish tissues a new antibiotic—ecmolin—which was first used in treating influenza.

Today the ecmolin-penicillin combination is effectively used against influenza, scarlet fever, pneumonia, acute catarrh of the respiratory organs, and in the case of diphtheria-carriers. Ecmolin induces penicillin to remain in the organism for 24 hours, which is much longer than if penicillin alone is administered. This, naturally, heightens its effect. A number of other antibiotics have been discovered and obtained, which might prove effective in treating many infectious diseases if they merely destroyed pathogens and were not harmful to man. Unfortunately, antibiotics which can be safely used are but few, and work is in progress to find such methods of chemical processing as would make the antibiotics harmless to man, animal and plants while preserving their antibacterial properties.

Chloromycetin has proved effective in treating dysentery, typhoid fever, typhus, and some other infections.

* Penicillin, streptomycin and other antibiotics do not always produce the desired effect. Certain strains of pathogenic microbes, as for instance, gonococci and tubercle bacilli, are resistant to a given antibiotic, which, therefore, is ineffective. In such cases a combination of two antibiotics, or of the antibiotic and some other medicine, is used.

But the extraction of chloromycetin from certain ray fungi, like the extraction of many other antibiotics, is a difficult and costly process. That is why Soviet scientists are seeking new methods of obtaining antibiotics, including chloromycetin, by purely chemical means (synthetic methods).

Soviet chemists have obtained two synthetic preparations—sintomycin and laevomycetin. The former is widely used for treating dysentery and dyspepsia in children. Some Soviet scientists prefer using laevomycetin for dysentery, considering it the better of the two.

Biomycin, another new antibiotic, gives a good effect in treating bacterial and amoebic dysentery, typhoid fever and cholera; it, too, is administered in combination with ecmolin. Biomycin can also be used in treating typhus, brucellosis and tularaemia. It exercises an inimical influence on staphylococci and streptococci, the organisms causing suppuration and sepsis. In several cases when penicillin and streptomycin failed to effect a cure, biomycin proved effective.

Subtilin, an antibiotic extracted from *Bacillus subtilis*, oppresses putrefactive organisms; if subtilin is added to perishable foodstuffs, they keep longer without undergoing any change in the flavour and general appearance. At present the possibility of its practical use is being extensively studied.

As actidione, an antibiotic obtained from an actinomyces variety, has proved highly poisonous for rats, it is used in fighting rodents.

Antimycin, an extract from another actinomyces variety, is more toxic for some insects, for instance, ticks, than chemical poisons.

A curious property of some antibiotics is their ability to accelerate the growth of piglings and poultry if introduced into their rations in minute doses (5 to 15 grammes per ton). Many antibiotics were tested for this property, including penicillin, streptomycin, chloromyce-

tin, bacitracin, aureomycin, and although it is yet early to speak definitely of their respective value for animal husbandry, still the best results as regards increase in weight and growth of piglings and poultry were obtained with aureomycin and terramycin.

The addition to fodder of antibiotics in combination with the recently discovered vitamin B_{12} yields excellent results; the addition of a combination of two or more antibiotics is more effective than one.

The use of antibiotics in practical animal husbandry is yet in its infancy. Antibiotics are expected to play a no less important role in this sphere than in medicine.

Chapter XII

DIRECTED VARIABILITY IN MICROBES

As far back as 1902 the Russian scientist D. Ivanovsky spoke in his famous *Mosaic Disease of Tobacco* about the influence the environment exercised on the growth of tobacco. He wrote: "By altering the external conditions of its growth I received a normal or abnormal shape of tobacco leaves at will." He pointed out that the influence of the environment could produce profound changes in the structure and functions of an organism. He was very careful in his conclusions, yet he ventured to say that "galls on oak leaves, or similar excrescences, often display an astonishingly complex and purposeful structure, and if we did not know that they were the result of an insect bite, we would never believe that a minute dose of a chemical substance introduced into plant tissue could alter its normal growth to such an extent."*

Mechnikov was more positive in stating that "it is precisely in the sphere of microbiology that the possibility of changing the nature of bacteria through changing their environment has been proved; the changes worked may become stable and hereditary."** In support of his idea

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* Quoted from S. Sobol, *The Evolutionary Views of D. I. Ivanovsky*, *Microbiology*, Vol. XIX, No. 6, 1950.

** From the speech Mechnikov made at Cambridge in 1909 at the celebration of C. Darwin's hundredth birthday, published in the collection of Mechnikov's articles *On Darwinism*, U.S.S.R. Academy of Science Publishing House, 1943, pp. 226-27.

Mechnikov cited examples from the famous discoveries of Pasteur and his pupil Emile Roux who had found a method of destroying the ability to form spores in the anthrax bacillus, which enabled them to prepare anthrax vaccine. Anthrax bacilli which had lost the ability to form spores transmitted this property to their offspring even if the latter were grown in natural and not specially modified conditions. As Mechnikov emphasized, that was a case of acquired properties and characters becoming hereditary.

The Pasteur vaccines mentioned in Chapter I were obtained as a result of the variability of anthrax bacilli which lost their virulence and the ability to form spores after being kept at a temperature between 108° and 109.4°F. for 20 days. The same anthrax culture kept at that temperature for 12 days lost spores but was weakened to an insignificant degree.

Another example of pathogen variability under the influence of environment is the transformation of virulent tubercular bacilli capable of causing tuberculosis into non-virulent, attenuated but still living rods. This was achieved by Calmette and Garin who worked at the Institut Pasteur in Paris. By completely altering the conditions of life of tubercular rods—the causal organism of bovine tuberculosis—which they grew for 13 years on a mixture of potato, glycerine and bile, the scientists made the rod lose almost all its virulence. But in order to achieve this, they had to make 230 passages in succession. From the weakened tubercle culture they prepared the *BCG* vaccine.

As the vaccine creates immunity against tuberculosis it is used for vaccinating infants. The vaccine is administered first of all to children of tubercular parents.

Chapter I also tells about Pasteur's method of preventing hydrophobia infection in men bitten by rabid dogs. This method, too, is based on the variability of organ-

isms. In the present instance it was the filter-passing virus of rabies which underwent variation.

By completely changing the conditions of life of the virus through drying the spinal cord of rabid rabbits for different periods of time, Pasteur obtained different degrees of weakening of the virus in the cords. The longer a cord was dried, the more was its virulence weakened, until it reached minimum strength after 14 days.

Changed conditions bring about a drastic change in the properties of the virus; its virulence is so weakened that it cannot cause the disease. Humans and animals inoculated a sufficient number of times with the weakened virus develop immunity against rabies.

Through changing the conditions of life of pathogens, Soviet scientists have succeeded in radically altering the properties of many of them, including the organisms of plague, tularaemia, brucellosis, and in weakening them to such an extent that they cannot cause disease. The introduction into a human organism of the weakened agent of some disease or other creates immunity against the disease. We see, then, that the production of vaccines from live but attenuated organisms is based on the variability of pathogens.

On the other hand, it is well known that when a pathogenic microbe somewhat weakened by adverse environmental conditions penetrates into the organism of a susceptible animal, the best medium for its development, it may regain its virulence and cause disease, or even death, of the animal.

The preceding chapter was devoted to antibiotics, particularly to penicillin which is obtained from certain varieties of the penicillium fungus. At first wild-growing strains of penicillium were used, but later conditions were created in laboratories which were best suited for the fungus to form the greatest quantity of substances going into the preparation of active penicillin. These were actually new strains of the fungus, so active as to yield 70

times as much penicillin as could be obtained from the original wild form.*

As stated in Chapter V, the production of alcohol is based on the action of certain types of yeast fungi upon various products containing sugar, from which wort is made. But foreign bacteria, too, get into the wort and interfere with the activity of the yeast and, consequently, with alcoholic fermentation.

Various substances, as for example sulphurous gas, inhibit the life and activity of bacteria, including those which interfere with fermentation. But at the same time the gas inhibits the normal development of alcohol yeasts. Specialists of the alcohol industry, however, have obtained such strains of yeasts which are not influenced by sulphurous gas, which has made it possible to pass the gas through wort and to oppress foreign bacteria, while the gas-resistant yeasts continue their fermentative action.

The usual temperature at which yeasts ferment sugars is 86°F. Soviet scientists have trained a yeast strain (strain XII *Saccharomyces cerevisiae)* to ferment carbohydrates under conditions of gradually increasing temperature. As a result, this strain trained to resist a temperature of 104°F. ferments carbohydrates at this temperature much better than the original strain.**

All the above facts of changed properties in microbes are examples of directed variability which is the result of conditions specially created by man for this or that species of micro-organisms with the aim of weakening or strengthening certain properties. This is necessary for obtaining better medical or veterinary preparations or for receiving maximum effect from microbial activity in industry or agriculture.

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* A. Imshenetsky, *Proceedings of the Conference on Directed Variability and Selection of Micro-Organisms*, U.S.S.R. Academy of Science Publishing House, 1952.

** *Ibid.*

We have but slightly touched on what has been achieved by Soviet scientists in studying the ability of micro-organisms to undergo directed changes. At a conference on directed variability of micro-organisms held at the U.S.S.R. Academy of Science in 1951, microbiologists noted the achievements of Soviet science in this sphere and mapped out ways for the further development and practical application of microbial variability.

EPILOGUE

This little book describes how the development of science has gradually drawn the curtain from the mysterious world of invisible organisms—microbes. The startling revelations which showed the important role of micro-organisms as causative agents of infectious diseases in man and animals have dispelled the myth of "damp, miasma-infected air," earthquakes, solar eclipses, comets and other "supernatural" phenomena being the causes of plague, cholera and other epidemics.

More and more scientists became interested in the life of microbes and they discovered that micro-organisms are important not only for agriculture, medicine, and veterinary science, but that they are responsible for the formation of fossil fuels and have important uses in food and other industries. During the short space of some 80 or 90 years microbiology—a versatile science—appeared, which unites an enormous number of microbiologists. Today nobody would dream of conducting research with such primitive means as Leeuwenhoek's magnifying-glass (although it was a great achievement in its time) or even the methods at the disposal of Pasteur, Mechnikov and other coryphaei of 19th-century science. Microbiology's further progress is closely linked with the development of biochemistry (and other sections of chemistry) and physics which has already provided microbiologists with

electron, phase-contrast and fluorescence microscopes. Work in the sphere of plant pathology cannot proceed without the assistance of agrobiology, and particularly, plant-breeding; medical microbiology requires a thorough knowledge of infectious diseases of man, and so on.

It would be wrong to divide all microbes into beneficent and harmful. Many of them are beneficent or harmful, depending on the conditions (natural or artificial) in which they develop. We may recall in this connection the observations of the activity of some microbes inhabiting the rhizosphere of plants. The bacteria contribute to the development of the plant when it excretes through the roots the substances necessary for the bacteria. If they do not receive the food they need, the symbiotic bacteria become parasitic and cause disease in the plant. Acetic bacteria are useful when they are employed in the production of vinegar but when they get into grape wine or any other foodstuff they cause putrefaction.

Proteolytic microbes (microbes decomposing proteins) are used in the production of certain ferments—proteases. Under natural conditions such microbes reduce animal remains to elementary mineral compounds suitable for plant feeding. The microbes, consequently, increase soil productivity. But if they have access to perishable products, such as fresh fish, meat, fowl, etc., they cause putrefaction, decay.

Last but not least, cellulose bacteria which occur universally in Nature, decompose the cellulose always present in large quantities in vegetable remains. These microbes which are always to be found in soil and on the ground, in sewage, and in many other places, produce, among other chemical compounds, combustible gases widely used in everyday life as well as in industry. Cellulose bacteria are used in industry for fermenting raw materials containing cellulose (wood wastage), in the production of alcohols and organic acids, and for testing

textiles and other cellulose materials. In all these cases cellulose bacteria are useful. But when they decompose paper, books, fabrics, fishing-nets, etc., they are the cause of spoilage.

Consequently, the examples given here of the useful or harmful action of different micro-organisms depending on conditions in which they develop, bear out the thesis of the decisive influence exercised on organisms by the environment.